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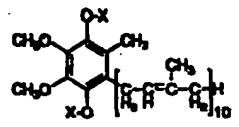
(54) Title: WATER SOLUBLE UBIQUINONE COMPOSITIONS, PRODRUGS, AND METHODS RELATING THERETO

(57) Abstract

Water soluble ubiquinone compositions and prodrugs are disclosed (I). Methods of their delivery and use in the amelioration of apoptosis are also disclosed.

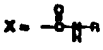
CoQ_{10} Source	Plasma (µg/ml)	Liver (µg/g)	Brain (µg/g)
Endogenous CoQ_{10}	50.9	5.0	8.7
Prodrug CoQ_{10}			
CoQ_{10} - carbonate - PHO	100.5 (+97%)	4.5	10.3 (+18%)
CoQ_{10} - ether - PHO	415 (+715%)	6.1	10.0 (+15%)
CoQ_{10} - (ether - PHO)$_2$	87.6 (+72%)	4.3	10.9 (+25%)



(I)

Ester Derivatives

Carbonate Derivatives



Carbamate Derivatives

Alkyl Ether Derivatives

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WATER SOLUBLE UBIQUINONE COMPOSITIONS, PRODRUGS, AND METHODS RELATING THERETO

Technical Field

The present invention generally relates to a water soluble ubiquinone compositions and prodrugs, methods of their delivery, and methods of their use in the amelioration of apoptosis.

Background of the Invention

Ubiquinones, specifically coenzyme Q_{10} (CoQ10), are highly hydrophobic molecules, which are essentially insoluble in aqueous solutions. Aqueous formulations are advantageous because such formulations allow for great flexibility in modes of administration, in particular, allowing for parental administration, while allowing for high concentrations of CoQ10 in lesser volumes and greater bioavailabilty. Intravenous formulations are particularly advantageous because of dependability, accuracy, and convenience of such formulations. Intravenous formulations also avoid gastric irritation which potentially accompanies oral administration of drugs. Intravenous formulations allow for continuous, as well as intermittent, drug therapy. Further, this method of administration is particularly valuable when the patient is unable to ingest an oral dosage due to, a malabsorption syndrome, an intolerance, lack of consciousness, or nausea. A fluid formulation, particularly a concentrated solution, of CoQ10 is of great value in formulating CoQ10 capsules. Further, highly hydrophobic drugs are poorly absorbed in the gastrointestinal tract. Due to the slow rates of absorption of CoQ10 from the gastrointestinal tract (6-10 h) and a period of about seven days to reach steady-state plasma levels, in most cases oral delivery cannot be used for the treatment of acute illness.

A clinically effective and usable aqueous formulation of CoQ10 should be stable at common ambient temperatures and remain essentially unchanged in dispersion characteristics for a period of at least a year. This is the approximate period which is required for preparation, analysis, shipment to distribution centers, shipment to and storage in hospitals for use and administration to a patient. The dispersion of solid particles of water-insoluble

CoQ10 in an aqueous medium renders the preparation of such a formulation very difficult. Solid particle dispersions have been attempted, but particles containing CoQ10 precipitate in the solution and redispersion methods such as stirring or shaking are not acceptable for clinical use. A second prominent difficulty associated with the insolubility of CoQ10 is having a formulation which on intravenous administration, does not lead to particle separation or precipitation within the bloodstream. Such a separation or precipitation would be detrimental to blood flow and potentially life threatening.

In an attempt to create a fluid formulation of CoQ10, others have primarily focused on the production of a fatty emulsion of CoQ10.

All of these formulations contain emulsifying agents. In fact, none of these CoQ10 formulations are free of detergents or surfactants. Further, because of the nature of the oil emulsion, the formulations provide limited bioavailability and concentrations of CoQ10 to the desired delivery sites in the body. Generally, oil formulations are highly viscous formulations with relatively low CoQ10 concentration and accumulate slowly into cell membranes; commonly no more than 10 mg/mL. More importantly, emulsions are slowly absorbed and accumulate at low levels in cells.

Despite the difficulties associated with CoQ10 administration, other CoQ10 formulations and methods of administration have been evaluated in clinical settings and demonstrate the potential and versatility of a pharmaceutical grade formulation for a broad spectrum of disorders. CoQ10 has been labeled a "breakthrough" drug in congestive heart failure - showing clinical benefit in 75% of patients (Greenberg and Frishman, J. Clin. Pharmacol. 30:596-608, 1990; Oda, Drugs Exp. Clin. Res. 11:557-76, 1985). CoQ10 has been used to combat the effects of muscular dystrophy, producing clinical benefit in a subpopulation of patients with Duchenne form (Folkers et al., Proc. Natl. Acad. Sci. U.S.A. 82:4513-6, 1985). CoQ10 has been successfully utilized to battle periodontal disease (Wilkinson et al., Res. Commun. Chem. Pathol. Pharmacol. 14:715-9, 1976). CoQ10 has been implicated in the reduction in toxicity of chemotherapeutic drugs, *e.g.*, cardiac toxicity of adriamycin (R. Ogura et al., J. Nutr. Sci. Vitaminol. 28:329-34, 1982). CoQ10 has been successfully implemented in the correction of drug-induced deficiencies. *e.g.*, psychotherapeutic, diabetes and beta-blocker drugs (Katsumoto and Inoue, Jpn. Circ. J. 47:356-62, 1983). CoQ10 has been used in immune restoration, *e.g.*, aging, AIDS, allergies (Suzuki et al., Jpn. J. Surg. 16:152-5, 1986; Folkers et al., Res. Commun. Chem. Pathol. Pharmacol. 38:335-8, 1982; Folkers et al., Biochem. Biophys. Res. Commun. 193:88-92, 1993).

A common denominator in many of these disorders is the presence of ongoing apoptosis. Apoptosis, or programmed cell death, is the major type of cell death in various physiological processes that lead to remodeling of developing embryos and adult cell turnover.

Apoptosis is also responsible for the removal of transformed or virus infected cells, and cells damaged via the release of reactive oxygen species, cytotoxic agents, macrophages and cytotoxic T-lymphocytes. Furthermore, apoptosis occurs in various pathological states, including such diverse diseases as AIDS and cancer, and likely occurs around cell injury sites like that surrounding myocardial infarction. Apoptosis is characterized by compaction of the nuclear chromatin into dense masses, convolution, fragmentation of the nucleus, and blebbing of the plasma membrane ultimately resulting in cell death.

There are a number of disorders which are caused or aggravated by the deleterious loss of cells due to apoptosis. For example, the accelerated loss of vital $CD4^+$ T-lymphocytes in HIV infected patients; the elimination of neurons, and other cell types, following ischemia and reperfusion of the brain; and the destruction of immune cells after exposure to ionizing or UV radiation in the treatment of neoplastic disorders. One common trigger of apoptosis in the etiology of these disorders is the involvement of oxidative stress, resulting primarily from the production of free radicals. Free radicals are highly reactive molecular species which interact with a wide variety of naturally occurring cellular molecules. When a free radical interacts with such cellular components, it produces a structural change which alters the normal functioning of the cell.

Free radicals are continuously generated in every cell as a product of normal cellular function (*e.g.*, oxygen metabolism and drug metabolism). However, if the concentration of free radicals in a cell exceeds a minimum toxic concentration (MTC), the structural and functional efficiency of lipids, proteins, carbohydrates, and nucleic acids--all vital components to the functioning cell--are altered. This "cellular damage" is a typical result of oxidative stress.

To combat cellular damage of this sort, warm-blooded animals have evolved a series of complex defense mechanisms to prevent free radical accumulation at levels exceeding the MTC. These defense mechanisms include a series of compounds that convert free radicals to stable compounds before the free radical can interact with a cell. These compounds are referred to as "free radical scavengers." The most important scavengers for radicals in membranes (lipid peroxides) are vitamin E and CoQ10. Recently, CoQ10 has been deemed to be the most important scavenger in a lipid environment (Stocker et al., Proc. Natl. Acad. Sci. U.S.A. 88:1646, 1991).

The availability of free radical scavengers throughout every cell in the body assures the stabilization and integrity of cell membranes. Generally, free radical scavengers are present in excessive concentrations in order to assure the cell a stable environment, although the absolute concentration of free radical scavengers present in cells varies from individual to individual. Further, free radical scavenger activity in an individual may be altered by a broad spectrum of influencing factors. The scavenging ability of the enzymes in a specific individual

is determined by measuring the rate at which the free radical scavenger can neutralize a given number of free radicals. This rate is designated the "free radical scavenger activity."

Under normal circumstances, the free radical scavenger activity maintains free radicals at concentrations well below the minimum toxic concentration ("MTC"). Thus, there is generally a substantial safety margin between free radical concentration and the MTC. However, a variety of factors may increase the free radical concentration, thereby endangering the patient. Such factors include considerations of genetics, nutritional status, exposure to drug therapy, drug metabolism, disease, and environmental factors. A change in any one of these factors may result in a failure of the body's defensive mechanisms and result in apoptosis.

In addition to free radical scavenging activity, cellular energy levels in the form of ATP or mitochondrial activity may be key in the prevention in cell death. For example, using a variety of leukemic cell lines with different levels of constitutively produced ATP, Smets et al. have reported that there is an inverse relationship between the level of cellular ATP and sensitivity to glucocorticoid-induced apoptosis (Blood 84(5):1613, 1994). Consequently, inhibitors of mitochondrial respiration potentiate cell sensitivity to glucocorticoid action. Therefore, methods that increase the production of ATP through mitochondrial activity would protect cells from agents that induce apoptotic cell death.

Due to the ability of CoQ10 to both act as a free radical scavenger in membranes and to offset the inability of cells to manufacture sufficient energy for cellular repair, CoQ10 possesses functional activities ideal for an inhibitor of apoptosis. Current ubiquinone formulations are limited in their ability to achieve desired bioavailability and solubility for clinical effectiveness, and thus it is apparent that there exists a need for new and additional methods and compositions which address and rectify the problem. The present invention fulfills this need, and further provides related advantages.

Summary of the Invention

One aspect of the present invention includes a water soluble ubiquinone prodrug comprising a ubiquinol substituted at at least one of positions C1 and C4 of the ubiquinol with a substituent independently selected from a solubilizing moiety and a targeting moiety. The ubiquinone prodrugs of this invention have C6 terpenoid side chains having from 1 to 12 isoprenoid units, preferably 6 to 10 units, and most preferably 10 units.

In one embodiment, the substituent solubilizing moiety is further coupled to a targeting moiety. In another embodiment, the substituent targeting moiety is further coupled to a solubilizing moiety. In one embodiment, the solubilizing moiety is a targeting group.

In one embodiment, the solubilizing moiety or targeting moiety is coupled to the ubiquinol through a linking group selected from carbamate, ether, ester, and carbonate linking groups.

In one embodiment, the solubilizing moiety is selected from an electronically charged and an electronically neutral solubilizing moiety. Representative electronically charged solubilizing moieties include moieties having a single charged group selected from sulfonate, carboxylate, phosphonate, and ammonium groups, as well as polycationic and polyanionic moieties including polypeptides. Representative electronically neutral solubilizing moieties include moieties having single solubilizing groups such as hydroxy, ether, amine, and thiol groups, as well as polyalcohol, polyether, and polyamine moieties. In a preferred embodiment, the solubilizing moiety is a polyether such as polyethylene glycol having a molecular weight in range of about 350 to about 6000, preferably in range of about 600 to about 3400, and most preferably in a range from about 1500 to about 2500.

In a preferred embodiment, the ubiquinone prodrug comprises a ubiquinone(50) linked to a polyethylene glycol 5000.

In another embodiment of this aspect of the invention, a targeting moiety is directed to a receptor selected from LDL receptors, asialoglycoprotein receptors, polyamine receptors, insulin receptors, transferrin receptors, and alpha-2-macroglobulin receptors. In a preferred embodiment, the targeting moiety is an asialoglycoprotein.

Another aspect of the present invention includes a pharmaceutical composition comprising a ubiquinone prodrug and optionally a pharmaceutically acceptable excipient or diluent.

Another aspect of the present invention provides a ubiquinone delivery composition comprised of a ubiquinone and a solubilizing agent, wherein the solubilizing agent is comprised of a lipophilic moiety coupled to a solubilizing moiety. Preferably, a lipophilic moiety is selected from a lipid or a phospholipid including fatty acids, fatty alcohols, and fatty esters. In a preferred embodiment, the lipophilic moiety is a sterol. Representative solubilizing moieties include electronically neutral solubilizing moieties, such as polyethers including polyethylene glycols having a molecular weight generally in the range of 350-6000, and preferably in the range of about 1500 to about 2500.

In another embodiment, the ubiquinone delivery composition includes a ubiquinone prodrug and a solubilizing agent. In a further embodiment, the ubiquinone delivery composition includes a ubiquinone, a ubiquinone prodrug, and a solubilizing agent.

In yet another aspect of the present invention, a pharmaceutical composition comprising a ubiquinone delivery composition and optionally a pharmaceutically acceptable excipient or diluent is provided.

Another aspect of the present invention is directed to a method of ubiquinone delivery to a warm-blooded animal utilizing a ubiquinone delivery composition. In another embodiment, the method utilizes a ubiquinone prodrug, and in still another embodiment, the

method utilizes a pharmaceutical composition comprising a ubiquinone prodrug or a ubiquinone delivery composition.

The present invention further provides methods for ameliorating apoptosis in a warm-blooded animal and/or a biological preparation. One aspect of the present invention, therefore, provides a method of ameliorating apoptosis comprising administering to a warm-blooded animal in need thereof a therapeutically effective amount of a water soluble ubiquinone prodrug or a ubiquinone delivery composition. The apoptosis may be associated with ischemia, a viral disorder, or a neurodengenerative disorder, for example.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth which describe in more detail certain procedures and/or compositions, and are hereby incorporated by reference in their entirety.

Brief Description of the Drawings

FIGURE 1 is a schematic representing the redox cycling of a ubiquinone. Formula 1 represents ubiquinone. Formula 2 represents ubiquinol;

FIGURE 2 is a formula representing ubiquinol with the coupling sites for the solubilizing moieties of the present invention indicated by X, as well as further exemplary cleavable linking groups suitable for use in the present invention;

FIGURE 3 is a photograph of a hematoxylin/eosin stained paraffin section of a brain of a rat in which brain ischemia was induced. This rat was not treated prior to or after the induction of ischemia. The rat and brain were prepared as outlined in Example 8 below. Briefly, brain ischemia was induced in this rat by bilateral occlusion of common carotid arteries for 8 minutes. The rat was perfused with 4% buffered formalin and the brain tissue was removed and embedded in paraffin 3 days after ischemia. The photograph shows significant neuronal cell death as indicated by apoptotic cells identified by dark cells designated 'a' in the photograph;

FIGURE 4 is a photograph of a rat brain prepared as described above for FIGURE 3, however this rat was treated with CoQ10 by administering a ubiquinone delivery composition of the present invention as outlined in Example 8. The photograph shows little or no neuronal cell death. In the photograph, non-apoptotic neuronal cells are designated 'b';

FIGURE 5 is a table that summarizes the percentage of apoptotic neuronal cells for rats treated with a representative ubiquinone delivery composition compared to untreated rats as a function of days after reperfusion following indication of brain ischemia by 8- and 12-minute arterial occlusion as outlined in Example 8; and

FIGURE 6 is a table that summarizes the bioavailability of CoQ10 in rats following administration by intraperitoneal injection of aqueous solutions a representative ubiquinone delivery composition and ubiquinone prodrugs. The table presents CoQ10 levels and the

percent increase in CoQ10 level following administration of the ubiquinone delivery composition and prodrugs.

Detailed Description

As noted above, the present invention provides water soluble ubiquinone derivatives and related compositions and methods for the treatment of a wide variety of disorders characterized by apoptosis. The present invention enables increased bioavailability of ubiquinones and facilitates administration of ubiquinones through increasing the water solubility of compositions effective in their delivery and providing ubiquinone prodrugs that are highly water soluble.

Functionally, ubiquinones are a group of lipid soluble benzoquinones some of which are involved in mitochondrial electron transport. Structurally, ubiquinones have a 2,3-dimethoxy-5-methylbenzoquinone nucleus and a variable terpenoid side chain containing from one to twelve mono-unsaturated *trans*-isoprenoid units (see the general structure below). The differences in properties among ubiquinones has been attributed to the difference in length of the terpenoid side chain. A dual nomenclature exists for these compounds and is based upon the length of the terpenoid side chain. A benzoquinone of this family is therefore properly referred to as either "coenzyme Q_n" where n is from one to twelve and designates the number of isoprenoid units in the side chain, or alternatively, "ubiquinone (x)" where x designates the total number of carbon atoms in the side chain and is a multiple of five. For example, the most common ubiquinone in animals has a ten isoprenoid side chain and is referred to as either coenzyme Q10 or ubiquinone (50).

Ubiquinones, including coenzyme Q10 (CoQ10), are essentially insoluble in aqueous media. This insolubility may be attributed to the long hydrocarbon isoprenoid side chain which bestows the molecule with its extremely lipophilic characteristics. These characteristics, among other effects, appear to be the source of the very slow absorption rates of the molecule. Pharmacokinetic data has demonstrated that intestinal absorption of ubiquinones is slow and inefficient in human subjects. By way of example, after administration of CoQ10, there is a lag time of about one hour before increased plasma levels of CoQ10 can be detected. A second absorption peak appears after about 24 hours. Approximately seven days of administration is required to achieve maximum steady-state plasma levels. Furthermore, absorption of orally administered CoQ10 is variable and generally in the range of only about 2-5 %.

In one aspect of the present invention, water soluble ubiquinone derivatives are disclosed. These ubiquinone derivatives are water soluble ubiquinone prodrugs that include a solubilizing moiety which provides the water soluble properties to the ubiquinone prodrug. As used herein, the term "prodrug" refers to a chemical compound that is converted *in vivo* to an active therapeutic agent by a metabolic process or biochemical or physiological condition.

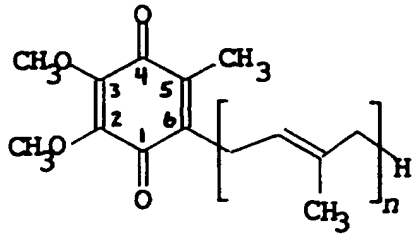
Thus, upon exposure to *in vivo* conditions including, by way of example, enzymatic activity, the ubiquinone prodrug is converted to a ubiquinone. In one embodiment of this aspect of the invention, a ubiquinone prodrug additionally includes a targeting moiety, which directs the compound to a specific site in the body or the cell.

In another aspect of the present invention, ubiquinone delivery compositions are provided. These ubiquinone delivery compositions include a ubiquinone and/or a water soluble ubiquinone prodrug in combination with a solubilizing agent. The solubilizing agent facilitates the effective administration of a ubiquinone or ubiquinone prodrug to a warm-blooded animal to elicit a therapeutic effect.

In another aspect of the present invention, a method of ubiquinone delivery utilizing a ubiquinone delivery composition is provided.

In another aspect of this invention, methods for ameliorating apoptosis, an example of a therapeutic effect, in warm-blooded animals through the administration of a ubiquinone or a ubiquinone prodrug are provided. In this method, delivery of the ubiquinone is accomplished either through the use of a water soluble ubiquinone prodrug or a ubiquinone delivery composition. Upon delivery, the antioxidant properties of the ubiquinone function to scavenge free radicals. These and other aspects of the invention are described in detail below.

The term "ubiquinone" includes compounds represented by the following general formula 1:



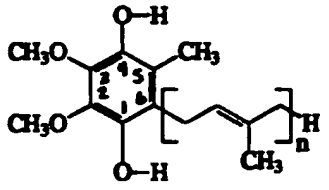
1

wherein typically n = 1-12, preferably n= 6-12, and most preferably n = 10. Ubiquinones utilized in the present invention may be isolated in nature where n = 6-10, or synthetically produced where n = 1-12 using any one of several methods, including, by way of example, those described in Ramasara, Coenzyme Q Biochemistry, Bioenergetics and Clinical Applications of Ubiquinone, G. Lenaz (ed.), John Wiley & Sons, New York, Ch. VI, pp. 131-144, 1985; Gibson and Young, Methods in Enzymology; and Fleischer and Packer (eds.), Academic Press, New York, pp. 600-609, 1978. One of ordinary skill in the art will appreciate that small changes may be made to the ubiquinone without altering the antioxidant function thereof.

Referring to the general ubiquinone formula above, small changes would include modifications of the methyl group at C5, the methoxy groups at C2 and C3, as well as modifications of the isoprenoid side chain at C6. Generally, these small changes would not significantly adversely alter the functional properties of the ubiquinone. More specifically, the modifications at one or more of these positions will not adversely affect the oxidation-reduction properties of the modified ubiquinone so as to significantly diminish its anti-oxidant characteristics. In addition, such small changes in the isoprenoid side chain will not adversely affect the lipophilic characteristics of the modified ubiquinone. Accordingly, small changes resulting from modification of the substituents of a particular ubiquinone's benzoquinone nucleus are included within the scope of the ubiquinones of the present invention provided that these changes do not significantly adversely affect the function of the ubiquinone either *in vivo* or *in vitro*.

Examples of small changes include modification or substitution of the C5 methyl group, the C2 and C3 methoxy groups, or the isoprenoid side chain with additional substituents such as lower alkyl groups having from one to six carbon including branched, cyclic and straight chain alkyl groups; aryl substituents including phenyl and substituted phenyl substituents; aralkyl substituents including benzyl and tolyl substituents; halogen substituents including fluoro substituents; oxygen substituents including hydroxy, lower alkoxy, ether, and ester substituents; nitrogen substituents including amino and amido substituents; sulfur substituents including thiol, thioether, and thioester substituents. In addition to substituting the C5 methyl group and/or the C2 and C3 methoxy groups with the above noted substituents, replacement of these groups with these substituents provides ubiquinones that are also included within the scope of this invention.

The term "ubiquinol" includes to a reduced form of ubiquinone (also known as "hydroquinone") represented by the following general formula 2:



2

As used herein, the term "ubiquinol," like the term "ubiquinone," encompasses synthetic and naturally occurring ubiquinols and the corresponding ubiquinols having small changes to their structure. The nature of the small changes to the ubiquinol structure are as described above for the ubiquinones of the present invention. The reversible reduction/oxidation shown in the interconversion of formulae 1 and 2 in FIGURE 1 is the

natural process involved in redox cycling for electron transport in mitochondria and in the characteristic antioxidant properties of the ubiquinone. Reduction of the quinone functional group of a ubiquinone is achieved chemically using any one of several reagents, including, by way of example, exposure to zinc in acetic acid, sodium borohydride, or lithium aluminum hydride. Reduction of the quinone may be confirmed by any one of several means, including, by way of example, observing a change in solution color from yellow to colorless and by spectroscopic methods including nuclear magnetic resonance spectroscopy. Reverse oxidation can be accomplished by any one of several means, including by way of example, exposure to air. Thus, preparation of a ubiquinone prodrug of the present invention starting from a ubiquinol is best conducted under an inert atmosphere, by way of example, an argon or a nitrogen atmosphere.

The term "pegylated" includes coupling polyethylene glycol (PEG) to a particular molecule. In the context of this invention, some of the water soluble ubiquinone prodrugs of this invention are pegylated CoQ10 derivatives. Coupling of a particular PEG to provide a ubiquinone prodrug may be achieved either by direct coupling to a ubiquinol or by coupling through a linker group or spacer group.

The term "solubilizing moiety" includes a moiety that, when covalently coupled to a ubiquinone (or coupled to a lipophilic moiety to produce a solubilizing agent), increases the water solubility of a ubiquinone (or a solubilizing agent). In one embodiment of the ubiquinone prodrugs of the present invention, the solubilizing moiety is coupled through a linking group.

The term "linking group" includes a moiety that serves to couple the solubilizing moiety to the ubiquinone moiety. As noted below, a linking group may also serve to couple a targeting moiety to either a solubilizing moiety or a ubiquinone moiety. Generally, the linking group results from a chemical reaction that occurs, for example, between a solubilizing (or targeting) moiety and a ubiquinone. Accordingly, the linking group includes a variety of functional groups. For example, the linking group may be an ester formed by reaction of the C1 phenolic hydroxy group of a ubiquinol with a carboxylic acid derivative of a solubilizing moiety. For the ubiquinone prodrugs of this invention, the linking group is cleaved *in vivo* and results in the release of a ubiquinone.

The term "targeting moiety" includes a moiety that, when covalently coupled to a ubiquinone or a solubilizing moiety, directs the molecule for uptake into a particular tissue or cell type generally through a receptor-mediated mechanism. One purpose of such a targeting moiety is to enhance processing of a ubiquinone, in particular water soluble forms of CoQ10, or to enhance accumulation into membranes of certain cells.

The term "solubilizing agent" includes a lipophilic moiety coupled to a solubilizing moiety which, when combined with a ubiquinone or derivative thereof, provides a ubiquinone delivery composition having increased water solubility.

The term "water soluble" includes the ability of a ubiquinone prodrug or ubiquinone delivery composition to dissolve in water or other aqueous milieu. Generally, a ubiquinone prodrug or ubiquinone delivery composition is considered soluble if it improves the solubility of a ubiquinone in water by about 10^3 to 10^8 and preferably by about 10^7 times; however, such improvement is not essential.

The term "apoptosis" includes programmed or apoptotic cell death. As noted above, apoptosis is the key mechanism associated with homeostatic regulation, a process by which cell death or suicide is naturally or deliberately invoked to produce cell turnover. Briefly, early stage events, such as disruption of the redox potential, activation of certain "death" genes, and exposure to mild toxic insult are part of several different complex mechanisms that ultimately funnel into the common late stage events. Such late stage events include protein synthesis and activation of magnesium and/or calcium-dependent endonucleases that lead to DNA fragmentation and, ultimately, apoptotic cell death.

The term "biological preparation" as used in this invention includes an ex vivo cell culture which may be suitable for introduction into a warm-blooded animal.

The term "ameliorate" or "amelioration" includes an inhibition of apoptosis such that the incidence of apoptosis is lower than that which would otherwise occur under the same or similar (i.e., control) conditions.

The term "ischemia" includes a temporary blockage of blood flow to a tissue that results oxygen deficiency and often cell death in affected tissues, including by way of example the brain, heart, spinal column, eyes, colon, and kidney. The term "reperfusion injury" refers to cell injury that occurs following restoration in blood flow in an ischemic tissue.

The term "treatment" includes reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, inhibition or elimination of the causative agent, or prevention of the infection or disorder in a subject who is free therefrom. A disorder is "treated" by partially or wholly remedying the deficiency which causes the deficiency or which makes it more severe. An unbalanced state disorder is "treated" by partially or wholly remedying the imbalance which causes the disorder or which makes it more severe. The subject may be a human patient or other warm-blooded animal.

In the context of the present invention, the term "blood-brain barrier" refers to the blood-brain barrier made up of brain microvessel endothelial cells characterized by tight intercellular junctions, minimal pinocytic activity, and the absence of fenestra. These characteristics endow these cells with the ability to restrict passage of most small polar blood-borne molecules (*e.g.*, neurotransmitters, including catecholamines and neuropeptides) and

macromolecules (*e.g.*, proteins) from the cerebrovascular circulation to the brain. The blood-brain barrier contains highly active enzyme systems as well, which further enhance the already very effective protective function. The blood-brain barrier is one of the greatest impediments to delivery of pharmaceuticals to the central nervous system. The transport of molecules to the brain is not determined solely by the molecular size but by the permeabilities governed by the specific chemical characteristics of the permeating substance and, additionally, by the presence of receptor-mediated transport mechanisms such as those associated with transferrin, insulin, and catecholamines. Thus, besides molecular size and lipophilicity, affinity of the substances to various blood proteins, specific enzymes in the blood, or the blood-brain barrier, will considerably influence the amount of a drug reaching the brain. Several mechanisms for crossing the blood-brain barrier are described below and others are known in the art.

One aspect of the present invention provides water soluble ubiquinone prodrugs which revert to a ubiquinone or a derivative thereof under *in vivo* conditions. The water soluble ubiquinone prodrugs of the present invention are comprised of a ubiquinol coupled to a solubilizing moiety.

In one embodiment of the present invention, a water soluble ubiquinone prodrug designed to revert to a ubiquinone under *in vivo* conditions is provided. In this embodiment, a solubilizing moiety is coupled to a ubiquinol at one or both of positions C1 and C4 (*i.e.*, the phenolic hydroxyl groups) of the ubiquinol, forming a water soluble ubiquinone prodrug. These prodrugs are cleaved spontaneously to ubiquinol and then oxidized to ubiquinone under *in vivo* conditions (*see* FIGURE 2).

Accordingly, in addition to prodrugs having solubilizing moieties substituted at both C1 and C4 positions of a ubiquinol, the prodrugs of this invention include ubiquinols substituted at position C1 with a solubilizing moiety and having a C4 phenolic hydroxy group, as well as ubiquinols substituted at position C4 with a solubilizing moiety and having a C1 phenolic hydroxy group.

The solubilizing moiety is linked to at least one of positions C1 and C4 of a ubiquinol to increase the aqueous solubility of the ubiquinone prodrug. Cleavage of the solubilizing moiety releases the ubiquinone, which represents the oxidized form of the ubiquinol. Thus, in effect, a ubiquinol linked through these positions to a solubilizing moiety increases the aqueous solubility of a ubiquinone.

In one embodiment of the present invention, a solubilizing moiety is coupled directly to a ubiquinol. In another embodiment, a solubilizing moiety is coupled to a ubiquinol through a linking group. Such linking groups are used to form prodrugs that are cleaved by *in vivo* conditions such as, by way of example, pH and enzymatic activity. Suitable prodrugs are cleaved, by way of example, by enzymes such as esterases, oxidases, reductases, peptidases, and demethylases. A cleavable linking group may be selected based on the desired stability of

the linkage between a ubiquinol and a solubilizing moiety. Suitable cleavable linking groups, include any group that may be cleaved *in vivo* including, by way of example, an ester, ether, carbamate, or carbonate group (*see* FIGURE 2). Other suitable cleavable linking groups include polymeric sequences of sugars, amino acids, or nucleic acids (*i.e.*, polysaccharide, polypeptide, nucleic acid sequences) that are cleaved by biological enzymes capable of cleaving these sequences. One of ordinary skill in the art will appreciate that the stability of ubiquinone prodrugs toward cleavage may be ascertained *in vitro* in various buffers and serum.

A ubiquinone prodrug of the present invention need not be cleaved directly to ubiquinone. It may require several enzymatic steps for a prodrug to be converted first to a ubiquinol, and then subsequently to a ubiquinone. The nature of the cleavage of a ubiquinone prodrug *in vitro* may be accomplished by any one of several means, including using a variety of liver homogenates. Evaluation of the physical and biological properties of the ubiquinone prodrugs before and after cleavage may be accomplished by any one of several means, including cyclic voltammetry, UV absorbance spectrophotometry, high pressure liquid chromatography, and mass spectrometry.

One aspect of the present invention includes a ubiquinone ester prodrug. This prodrug is readily cleaved by any one of several enzymes, including esterase enzyme present in the blood serum. Due to the nature of the ubiquinol, a ubiquinone ester prodrug is a readily cleaved derivative and, thus, is particularly suited for cases where a quick release of a ubiquinone is desired. A ubiquinone ester prodrug may be formed using any one of several means, including using an esterifying agent such as, by way of example, an acid chloride, an activated acid ester (*e.g.*, N-hydroxysuccinimide and tetrafluorophenyl esters), and an anhydride.

Other aspects of the present invention include a ubiquinone carbonate prodrug and a ubiquinone carbamate prodrug. These prodrugs are acid sensitive and are be cleaved *in vivo* in the endosomes and lysosomes. Specifically, these ubiquinone prodrugs are endocytosed by components of the reticular endothelial system (*e.g.*, Kupfer cells) or hepatic parenchyma cells and subsequently processed through intravesicular pathways of the cells. In polar cells such as liver parenchyma cells, one pole of the cell may be responsible for uptake of the ubiquinone. Following conversion to the prodrug, the resulting ubiquinone associates with LDL cholesterol, low density lipoprotein cholesterol complex, for transport in the blood serum.

Ubiquinone carbonate prodrugs may be formed using any one of several means including the use of a carbonate forming agent such as, by way of example, a carbonyl chloride. For example, reaction of a carbonyl chloride of a ubiquinol with an alcohol form of a solubilizing moiety produces a carbonate linkage. Conversely, reaction of a ubiquinol with a carbonyl chloride of a solubilizing moiety similarly provides a carbonate linkage.

Ubiquinone carbamate prodrugs may be formed using any one of several means including the use of a carbamate forming agent such as, by way of example, an isocyanate reagent. The preparation of representative ubiquinone prodrugs having a carbamate linking group is described in Example 11.

Yet another aspect of the present invention includes a ubiquinone ether prodrug. Ubiquinone ether prodrugs are formed by any one of several means including the use of an alkylating agent such as an alkyl halide, a benzylic halide, an alkyl sulfonate (*e.g.*, tosylates, thesylates, triflates), and a benzylic sulfonate. The preparation of representative ubiquinone prodrugs having an ether linkage is described in Examples 10 and 12.

In another embodiment of this aspect of the present invention, the prodrugs are formed stepwise. In a first step, a spacer group containing one of these functional groups is reacted with a ubiquinol and, in a subsequent step, a solubilizing moiety (or targeting moiety) is coupled to the spacer group on the modified ubiquinol. Typically spacer groups are bifunctional reagents capable of reaction with, for example, both a ubiquinol and a solubilizing or targeting agent. Suitable spacer groups are known in the art and include dicarboxylic acid groups such as subaric and sebacic acids, and amino acids such as 4-aminobutyric acid, among others. An advantage of the spacer group is to provide additional length between, for example, the solubilizing group and the ubiquinol to relieve steric congestion that might otherwise impede coupling to or cleavage from the solubilizing or targeting moiety to the ubiquinol. One of ordinary skill in the art will appreciate that a solubilizing moiety (or targeting moiety) may be coupled directly to a ubiquinone through a linking group or a spacer group as long as the desired characteristics of the molecule are not compromised by the additional atoms.

A suitable solubilizing moiety includes any hydrophilic moiety which increases the solubility of the prodrug over that of a native ubiquinone and is cleavable, or is coupled to ubiquinone by a linker cleavable, *in vivo*. The solubility of the compound may be determined by "Handbook of Solubility Parameters and Other Cohesion Parameters" by AFM Benton, CRC Press, 1983. Hydrophilic moieties are polar moieties which may be either charged (*i.e.*, ionic) or electronically neutral. It should be appreciated that, if more than one solubilizing moiety is utilized, the hydrophilicity of the moieties may be additive.

Suitable hydrophilic moieties include electronically neutral solubilizing moieties. These moieties contain one or more polar functional groups (*i.e.*, groups which contain atoms of differing electronegativities such as organic compounds containing nitrogen, oxygen, and sulfur) that increase their hydrophilicity. Typically, these neutral solubilizing moieties contain functional groups capable of forming hydrogen bonds with water. Such hydrogen bonding groups include ether (-O-), hydroxy (-OH), amino ($-NR_2$, -NHR, $-NH_2$), and thioether (-S-) and thiol (-SH) groups. Other polar functional groups which may serve as hydrophilic

moieties include carbonyl containing groups such as acids, esters, amides, ketones, and aldehydes. Moieties which contain multiple polar functional groups are more hydrophilic than those moieties which contain a single polar functional group. Suitable moieties include, by way of example, polyalcohol, polyamine, polyether and polypeptide moieties. Polyalcohol moieties include, by way of example, glycols, glycerols, and polysaccharides including glucose, fructose, galactose, idose, inositol, mannose, tagatose, N-methylglucamine, and dextran moieties. Polyether moieties include, by way of example, polyethylene glycol, ethoxyethanol, and ethoxyethoxyethanol moieties. Polyamine moieties include, by way of example, spermine and spermidine moieties. Polypeptide moieties include peptides that contain electronically neutral amino acid residues.

In a particularly preferred embodiment, a solubilizing moiety is a polyethylene glycol. Generally, suitable polyethylene glycols include those with a molecular weight in the range of about 350 to about 6000; typically with a molecular weight in the range of about 600 to about 3400; and preferably in the range of about 1500 to about 2500 and, even more preferably, selected from polyethylene glycol 2000, polyethylene glycol 3400, and polyethylene glycol 5000 (Aldrich Chemical Co., Milwaukee, Wisconsin and Shearwater Polymers, Inc., Huntsville, AL). The polyethylene glycols (PEGs) noted above are designated by their approximate molecular weights (e.g., about 1500), and more specifically their approximate molecular weight ranges (e.g., about 1500 to about 2500). Commercially available PEGs are available as mixtures of PEGs having designated average molecular weights (*e.g.*, polyethylene glycol 20,243-6 from Aldrich Chemical Co. has average M_n *ca.* 1,500). These PEGs are available as mixtures having average molecular weights due to the polymeric syntheses from which the PEGs are prepared. The present invention includes PEG mixtures having designated average molecular weights, and also includes PEG and PEG containing compounds that are homogeneous and have discrete molecular weights.

Suitable electronically charged solubilizing moieties include those moieties which become either formally negatively or positively charged in water. Accordingly, electronically charged solubilizing moieties include anionic and cationic moieties, polyanionic and polycationic moieties, and moieties that contain both anionic and cationic species. Suitable negatively charged moieties include acid anions resulting from the dissociation of acids in water. For example, carboxylic acids ($-CO_2H$) dissociate to form negatively charged carboxylate ions ($-CO_2^-$) (pH greater than 5). Other stronger acids such as phosphoric ($-PO_3H_2$) and sulfonic ($-SO_3H$) acids ionize to form phosphonate ($-PO_3^{2-}$) and sulfonate ($-SO_3^-$) anions, respectively (pH greater than about 2). Other more weakly acidic moieties such as phenols and thiols may also dissociate to form their corresponding anionic derivatives which are water solubilizing (depending upon the pH of the aqueous solution). Basic moieties may become formally positively charged moieties in water. These moieties become highly

water soluble through protonation in aqueous solution. For example, at pH less than about 5, amines ($-NR_2$, $-NHR$, $-NH_2$, where R is an alkyl or aryl group) become ammonium ions ($-NHR_2^+$, $-NH_2R^+$, $-NH_3^+$), all of which are highly water solubilizing moieties. Quaternary ammonium moieties ($-NR_3^+$) are extremely water solubilizing (at all pHs).

Suitable electronically charged solubilizing moieties include polypeptides containing cationic, or anionic, or both cationic and anionic amino acid residues. Other polycationic moieties include, for example, spermine, spermidine, and polylysine moieties.

Water solubility of a ubiquinone prodrug may be evaluated by any one of several means, including, by way of example, simply combining the derivatives with an aqueous medium and observing the solubility at various temperatures. For the polyethylene glycol containing ubiquinone prodrugs of the present invention, the water solubility is determined by the components of the prodrug, namely, the lipophilic nature of the particular ubiquinone and the hydrophilic nature of the particular PEG solubilizing moiety. Generally, the longer the terpenoid side chain of a ubiquinone, the greater is its lipophilicity thus requiring a PEG solubilizing agent having a greater molecular weight. For example, while CoQ2 (i.e., ubiquinone(10)) may be readily water solubilized by a PEG 900 solubilizing moiety, CoQ10 is best water solubilized by a PEG solubilizing moiety having a molecular weight of 1500 or greater. Thus, the water solubility of a particular ubiquinone prodrug is controlled by the nature of the solubilizing moiety.

By way of example, a ubiquinone prodrug containing greater than about 10% by weight PEG 5000 is highly hygroscopic and forms a gel. A ubiquinone prodrug that contains less than about 10% by weight PEG 5000 forms a solution. Either of these prodrugs can be further diluted with water to provide an aqueous solution of the prodrug. For prodrugs containing, for example, PEG 2000, gel formation does not occur until the weight percent of the PEG is greater than 10%.

The syntheses of representative ubiquinone prodrugs derived from CoQ10 and containing PEG 5000 as a water solubilizing moiety are described in Examples 10, 11, 13, and 14. For example, these ubiquinone prodrugs of Examples 11 and 12 are soluble in water to the extent of at least 40 mg/mL which corresponds to a concentration of CoQ10 in water of about 10 mg/mL. In contrast to this result, CoQ10 has been reported to be water insoluble (Ondarroa et al., Bioscience Reports, 6:783, 1986). The concentration of ubiquinone prodrug in water noted above represents a usable concentration with regard to the methods of administration described below. The high water solubility of the ubiquinone prodrugs of the present invention provides a great improvement in the effective administration and delivery of ubiquinones in general, and CoQ10 in particular.

Alternatively, water solubility may be ascertained by dissolving the derivative in water, stirring the solution and allowing the solution to stand at room temperature for about 24

hours. The solution is then centrifuged and the resultant aqueous layer analyzed using high pressure liquid chromatography (HPLC). The HPLC analysis may be conducted isocratically using acetonitrile as the solvent on a LiChrospher 100, C-18 column (5 μM, 125 x 4 mm) using a flow rate of 2 mL/min. Under these conditions, the coenzyme Q10-containing product has a retention time of 7.6 min.

The quantitation of the coenzyme Q_{10}-containing solution may be accomplished by HPLC using UV detection at about 254 nm. In the quantitation, an aqueous solution of a coenzyme Q10 derivative is prepared and analyzed by HPLC as described above. A series of aqueous solutions of coenzyme Q10 derivatives of known concentration are prepared and analyzed by HPLC. The results of these HPLC analyses are then used to construct a standard curve where the concentration of the coenzyme Q10 standard is plotted against the HPLC signal for the standard. Once such a standard curve has been constructed, aqueous solutions of coenzyme Q10 derivatives may be similarly analyzed and the concentration of the derivative in the solution determined.

Alternatively, the water solubility of a ubiquinone prodrug (*i.e.*, the aqueous concentration of a coenzyme Q10 derivative) may be determined directly by absorbance spectroscopy. Briefly, a known amount of a ubiquinone prodrug is dissolved in a known amount of water to provide an aqueous solution of known concentration (*e.g.*, 10 mg prodrug/10 mL water). The absorbance of this solution (or dilutions of the solution) is then measured by a UV absorbance spectrophotometer. The absorbance of the solution of known concentration provides the prodrug's absorptivity (*e.g.*, a 10 mg/10 mL solution produces an absorbance of 0.95). Once the prodrug's absorptivity has been determined in this manner, the concentration (or the amount of prodrug in the solution) of subsequent aqueous solutions of the prodrug may be determined by measuring the absorbance of the solution. A ubiquinone prodrug may be then cleaved to a ubiquinol, using any of the *in vivo* conditions outlined above, then the ubiquinol may be allowed to revert to a ubiquinone and the absorptivity measured in solution. The absorptivity may then be compared to the standard created above.

Increased concentrations of water soluble ubiquinone prodrugs will result in more viscous solutions. One of ordinary skill in the art will appreciate that different viscosities will have different applications. For example, at high concentrations, certain water soluble ubiquinone prodrugs may form a gel. This formulation is suitable for use for some capsules and in particle dissociation in inhaler systems for pulmonary delivery.

In a preferred embodiment, a ubiquinone (50) is used to form a ubiquinone prodrug of the present invention. In a particularly preferred embodiment of the present invention, a ubiquinone (50) is coupled to a polyether solubilizing moiety such as a polyethylene glycol with a molecular weight in the range of about 350 to 6000; typically with a molecular weight in the range of about 600 to about 3400; and preferably in the range of about 1500 to about

2500 and, even more preferably, selected from polyethylene glycol 2000, polyethylene glycol 3400, and polyethylene glycol 5000.

In another embodiment, ubiquinone prodrugs detailed above are additionally coupled to a targeting moiety. The targeting moiety is preferably coupled to the ubiquinone prodrug via a solubilizing moiety.

Suitable targeting moieties include any moiety which will direct the ubiquinone prodrug to a desired cell surface receptor. In a preferred embodiment, a targeting moiety has an affinity for a cell surface receptor of within 100-fold, and more preferably, within 10-fold, of the affinity of the natural ligand for the receptor. Covalent attachment of a targeting moiety should not significantly hinder the ability of the targeting moiety to specifically bind the cell surface receptor. Other moieties may also be present, so long as they do not interfere with either the targeting or the solubilizing moieties. Optimal attachment of the targeting moieties to ubiquinone may be determined by comparing the affinity of binding of the ubiquinone prodrug with a free targeting moiety in assays of inhibition of binding. This, and other suitable techniques, are described in detail in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1989.

The targeting moiety is covalently attached to the ubiquinone prodrug by any suitable means known in the art, including direct covalent linkage, through a solubilizing moiety, or through the use of a linking group. In the context of this invention, the targeting moiety is coupled to the solubilizing moiety, or alternatively, to one of the C1 or C4 positions (*i.e.*, the phenolic hydroxy groups) of the ubiquinol either directly or through the use of a linking group. Coupling of a targeting moiety through a solubilizing moiety is preferably of a nature which resists cleavage by the enzymatic and low pH conditions normally encountered *in vivo*. Coupling of a targeting moiety directly to a ubiquinone or ubiquinone prodrug is preferably of a nature which does not resist cleavage *in vivo*. The ability to resist cleavage may be detected by any means known in the art, including exposing the ubiquinone to enzymes at low pH and measuring release of the targeting or solubilizing moiety using techniques known in the art. A preferred linking group is an ether.

Suitable targeting moieties include ligands such as proteins, peptides, and non-proteinaceous molecules. Representative examples of suitable targeting moieties include antibody and antibody fragments; peptides such as bombesin, gastrin-releasing peptide, cell adhesion peptides, substance P, neuromedin-B, neuromedin-C and metenkephalin; hormones, including EGF, alpha- and beta-TGF, estradiol, neurotensin, melanocyte stimulating hormone, follicle stimulating hormone, luteinizing hormone, and human growth hormone; proteins or peptides corresponding to ligands for known cell surface receptors, including low density lipoproteins, transferrin, vitamin B_{12}, and insulin; fibrinolytic enzymes; and biological response modifiers, including interleukin, interferon, erythropoietin and colony stimulating factor.

Moreover, analogs of the above targeting moieties that retain the ability to specifically bind to a cell surface receptor are suitable targeting moieties.

In a preferred embodiment, a targeting moiety is a ligand directed to a receptor selected from LDL receptors, polyamine receptors, insulin receptors, and alpha-2-macroglobulin receptors.

In a particularly preferred embodiment, a targeting moiety is selected which directs the ubiquinone prodrug to the liver. Such targeting moieties include, by way of example, targeting moieties which direct binding to an asialoglycoprotein receptor, including triantennary galactosides and arabinogalactan. In a particularly preferred embodiment, a targeting moiety is a lactosylated, galactosylated, mannosylated or glycosylated dextran-based ligand for lectin-like carbohydrate receptors such as the asialoglycoprotein receptor or galactose receptor.

In another preferred embodiment, a targeting moiety is directed to the blood-brain barrier. Suitable targeting moieties for this purpose include, by way of example, ligands directed to receptors on cells which mediate blood-brain barrier transport, such as endothelial cells. Such receptors include, by way of example, transferrin receptors, and low density lipoprotein receptors.

The targeting characteristics of a ubiquinone prodrug may be ascertained by any one of several means including, by way of example, utilizing radiolabeled derivatives to track pharmacokinetics of targeted molecules (see, *e.g.*, Tomono et al., Int'l J. of Clin. Pharm., Therapy and Toxicology 24(10):536-541, 1986).

As noted above, another aspect of the present invention provides a ubiquinone delivery composition. One embodiment of the present invention provides a ubiquinone delivery composition comprised of a solubilizing agent and a ubiquinone. Another embodiment provides a ubiquinone delivery composition comprised of a ubiquinone prodrug, a solubilizing agent, and a ubiquinone. Yet another embodiment provides a ubiquinone delivery composition comprised of a ubiquinone prodrug and a ubiquinone. These and other embodiments are described in detail below.

Suitable solubilizing agents include compounds having a lipophilic portion or moiety and a solubilizing portion or moiety such that, when combined with a ubiquinone or derivative thereof, a water soluble composition is formed. Hence, the solubilizing moiety of the solubilizing agent must be sufficiently hydrophilic to solubilize the solubilizing agent and associated ubiquinone or derivative thereof in aqueous medium. Further, the lipophilic moiety must be capable of associating with the lipophilic portion of a ubiquinone or derivative thereof. Suitable lipophilic moieties include lipids and phospholipids, including by way of example, fatty acids, fatty esters, and fatty alcohols. Suitable fatty acids include branched and straight chain fatty acids having from about 8 carbons to about 30 carbons including saturated

and unsaturated fatty acids. Representative fatty acids include linoleic, linolenic, and oleic acids. Representative fatty alcohols also include branched and straight chain fatty alcohols having from about 8 carbons to about 30 carbons including saturated and unsaturated fatty alcohols. Suitable fatty alcohols include sterols. A preferable sterol is cholesterol. Lipophilic moieties may be comprised of more than one fatty acid or a combination of a variety of lipophilic moieties (*e.g.*, linoleic-linoleic-polyethylene glycol). In the context of the solubilizing agents of the present invention, suitable solubilizing moieties include the electronically neutral solubilizing moieties described above.

Suitable solubilizing agents include lipophilic moieties comprising fatty alcohols, fatty esters, and fatty acids coupled to electronically neutral solubilizing moieties such as PEGs, polysaccharides, and polypeptides.

In one aspect of the present invention, a solubilizing moiety may be coupled to a lipophilic moiety either covalently or through a lipophilic linking agent. Any one of several suitable homo- or hetero-bifunctional lipophilic linking agents may be utilized including, by way of example, suberic acid, succinic acid, and sebacic acid. Covalent coupling of the solubilizing moiety and the lipophilic moiety is of a nature which allows cleavage in the enzymatic and low pH conditions normally encountered *in vivo*. The ability to resist cleavage may be detected by any means known in the art, including exposing the solubilizing agent to enzymes at low pH and measuring release of the solubilizing or lipophilic moiety using techniques known in the art.

Covalent coupling of the solubilizing moiety and the lipophilic moiety should not significantly hinder the hydrophilic character of the solubilizing moiety. Other moieties may also be present, so long as they do not interfere with the characteristics of either the solubilizing or the lipophilic moieties. Optimal attachment of the two moieties may be determined by comparing the solubility of the various solubilizing agents thus produced. Solubility can be determined by the methods described in detail above.

In a preferred embodiment, a solubilizing agent is comprised of a cholesterol coupled to any one of the above-noted electronically neutral solubilizing moieties. In a particularly preferred embodiment, a solubilizing agent is comprised of a cholesterol pegylated through a sebacic acid linker.

A solubilizing agent and a ubiquinone (or derivative thereof) are combined under suitable conditions and for a time sufficient to produce a water soluble ubiquinone delivery composition. Briefly, by way of example, a ubiquinone and a solubilizing agent are dissolved in an organic solvent, water is added, the liquid is evaporated to remove organic solvent, and water or a buffered aqueous solution, *e.g.*, phosphate buffered saline, is added. The organic solvent may be any one of several water miscible solvents including alcohols such as methanol, ethanol, and propanol, dimethylsulfoxide, and, preferably, tetrahydrofuran. The liquid may be

evaporated at ambient temperature by any one of several means, including vacuum or a stream of an inert gas. The molar ratio of ubiquinone to solubilizing agent generally should be in the range of about 1:2 to about 1:10; preferably, the molar ratio is about 1:3. After evaporation of the lipid the ubiquinone/solubilizing agent formulation is dissolved in water to a required concentration of ubiquinone. The ubiquinone content may be ascertained by any one of several means including spectrophotometrically following dilution in ethanol from the extinction coefficient at 275 nm of 14,200.

Another aspect of the present invention provides for the administration of a ubiquinone, via a ubiquinone prodrug or a ubiquinone delivery composition, to a warm-blooded animal or a biological preparation in an amount sufficient to ameliorate apoptotic cell death. In the context of this invention, warm-blooded animal includes human. Briefly, as noted above, apoptosis is associated with, among other causes, oxidative stress resulting from the production of free radicals. Free radicals or oxidants, such as superoxide radicals, hydrogen peroxides, hydroxyl radicals and lipid peroxides, are intimately intertwined in the etiology of many disease states (Saltman, Semin. Hematol. 26:249, 1989). As noted above, the mammalian cell has developed a wide variety of free radical scavengers to combat the damage wrought by free radicals. But not all free radical scavengers act in the same manner. By way of example, some free radical scavengers (e.g., carotenoids) are readily diffusible in the cellular environment and, thus, must be constantly replenished in order to maintain consistent activity.

Another class of free radical scavengers integrate into the lipid membranes, thus maintaining a relatively constant presence in the cell. This class includes lipophilic free radical scavengers, by way of example, the antioxidants alpha-tocopherol (Vitamin E) and CoQ10. CoQ10 has demonstrated its antioxidant effect on emulsions of arachadonic acid and isolated mitochondrial membranes subjected to peroxidative insult (Mellors and Tappei, J. Biol. Chem. 241:4353, 1966; Battino et al., Biomedical and Clinical Aspects of Coenzyme Q, Folkers et al. (eds.), Elsevier, Vol. 6, p. 181-190, 1991).

Ubiquinones are particularly well suited for treating apoptosis. In the first instance, ubiquinones are superior antioxidants. For example, CoQ10 is oxidized before alpha-tocopherol or carotenoids in disarming the toxic products of low density lipoproteins (LDL). Furthermore, the concentrations of the hydroperoxides of phospholipids and cholesterol begin to increase once the available CoQ10 has been consumed, despite the constant presence of alpha-tocopherol and carotenoid concentrations (Stocker et al., Proc. Natl. Acad. Sci. U.S.A. 88:1646, 1991).

Secondly, ubiquinones regenerate other antioxidants to their active form by donating electrons. This is exemplified by the following reaction with alpha-tocopherol:

$$\text{tocopherol-O}\cdot + \text{CoQH}_2 \rightarrow \text{tocopherol-OH} + \text{CoQH}\cdot$$

This electron donating feature (believed to be due to the favorable redox potential of ubiquinol, a ubiquinone intermediate) helps maintain high levels of antioxidants within a variety of compartments within the cell.

Lastly, ubiquinones are essential for supplying the cell with energy. Ubiquinones play an integral role in transporting electrons in the respiratory chain by coupling the transfer and translocation of electrons through the hydrocarbon osmotic carbon domain of the inner mitochondrial membranes. The location of ubiquinones within the electron transport chain is between the flavoproteins and cytochrome b-c_1 complex. Ubiquinone functions as a redox carrier between the two flavin-FeS dehydrogenases and the cytochromes b and the later cytochrome c_1. In addition to its catalytic function, ubiquinone has some regulatory effects on succinate dehydrogenase (Rossi et al., Eur. J. Biochem. 16:508, 1970), NADH dehydrogenase (Gutman et al., Biochemistry 10:2036, 1971), and the cytochrome b-c_1 complex (Nelson et al., Biochem. Biophys. Res. Commun. 44:1312, 1971). The energy generated by the respiratory transport system is in the form of ATP, the ubiquitous energy molecule of the cell. Energy is generated by breaking a high energy phosphate bond ($ATP = ADP + P_i$), supplying energy for a variety of chemical synthetic reactions. Most disease states involve excessive energy consumption within the cell resulting in ATP depletion. With regard to apoptosis, an abundant energy supply is necessary to synthesize molecules involved in and carry out cellular repair caused by various indicators of apoptosis. Since a damaged cell is a signal for the apoptotic process, repair with non-lethal damage can offset the apoptotic cascade. These three characteristics render ubiquinones particularly suitable for use in amelioration of apoptosis in warm-blooded animals, and biological preparations.

However, as noted above, ubiquinones are poorly soluble in aqueous solutions, and as a result are poorly assimilated *in vivo*. Consequently, the limited bioavailability of ubiquinones decreases their utility as therapeutic agents.

In one aspect of the present invention, the ubiquinone prodrugs and delivery compositions are utilized for the treatment of apoptosis associated with ischemia. Following ischemia there is a significant increase in free radical production. The initial damage to neuronal tissue is thought to result from the peroxidation products with subsequent damage from secondary radicals. Eventually the metabolic and synthetic pathways are damaged to such an extent that the cell cannot make sufficient repairs and dies. The invention includes ubiquinone prodrugs and ubiquinone delivery compositions, which pass through the blood-brain barrier and decrease or prevent cell damage during or after ischemia by decreasing or preventing ATP depletion, thus stabilizing the perturbed redox status of the cells and tissue. By way of example, ischemic injuries may occur to the central nervous system as a result of

conditions such as stroke, cerebral pressure, cerebral infarction, physical trauma, such as head and spinal injuries, oxygen deprivation, cerebral hemorrhage, such as arachnoid hemorrhage and subdural hemorrhage, and chemical trauma. Ischemic injuries may also occur as a result of cardiovascular disorders such as angina infarction, cardiac arrest, congestive heart failure, cardiac arrhythmias, arterial hypertension, arteriosclerosis, occlusion of the aorta, cardiomyopathy and transient ischemic attacks. Other disorders which may result in ischemic injury include reanastomosis, frostbite, colic, and founder.

In another aspect of the present invention, ubiquinone prodrugs and delivery compositions are administered to treat disorder or damage associated with exposure to high pressure oxygen or oxygen radical enriched environments. By way of example, such conditions include ocular damage in babies placed on high oxygen respirators, drug and alcohol induced damage to the CNS or other organs, and damage resulting from ionizing radiation or photo-oxidation.

In yet another aspect of the present invention, ubiquinone prodrugs and delivery compositions are administered to ameliorate apoptosis in a warm-blooded animal suffering from, or susceptible to, a viral disorder. In one embodiment, ubiquinone prodrugs and delivery compositions are administered to a biological preparation to ameliorate apoptosis in a warm-blooded animal suffering from, or susceptible to, a viral disorder. Such viral disorders include, by way of example, AIDS (Fauci, Science 262:1011, 1993; Ameisen, Immunol. Today 13:388, 1992; Gorla et al., AIDS Research and Human Retroviruses 10(9):1097, 1994) and hepatitis. The etiology of HIV infection involves the gradual loss of T-cell function characterized by the failure of T-cells to proliferate in response to various mitogens and recall antigens. Ubiquinones play a changing role over the course of the disease.

The association between a viral infection, in the case of AIDS, and apoptosis can be identified by any one of several *ex vivo* assays in which lymphocytes, preferably T-lymphocytes, are isolated from the warm-blooded animal and assayed for apoptosis. Within one embodiment, an assay is used which utilizes detection of DNA strand breaks in apoptotic cells using the terminal deoxynucleotidyl transferase method (*e.g.*, Gorczyca et al., Cancer Research 53:1945, 1993). Briefly, a blood sample is drawn by vein puncture and white blood cells are isolated by Ficoll-Hypaque centrifugation. The cells are washed in phosphate buffered saline and T-cells are then purified using a variety of techniques available, such as cell sorting by FACS or by affinity chromatography in which T-cells are bound by specific antibodies to a matrix and non-T-cells are eluted through the column. Next, the bound cells are eluted and checked for purity by fluorescent labeled antibodies on a sample of the population. For detection of apoptosis, cells are ethanol fixed and resuspended in a sodium cacodylate buffer. Biotinylated nucleosides (*e.g.*, biotin-16-dUTP) are added to the fixed cells along with avidin-FITC. After incubation and washing, the cells are examined by cytometry

for incorporation of the labeled nucleoside into the fragmented DNA of apoptotic cells. In order to visualize significant numbers of cells programmed to undergo apoptosis, lymphocytes can be activated with mitogens (*e.g.*, PHA), bacterial superantigens, or anti-CD3 monoclonal antibodies before the fixation process. Such activation results in the death of cells destined to undergo apoptotic cell death that would normally not be visualized in a short term assay.

In another aspect of the present invention, ubiquinone prodrugs and delivery compositions may be administered to ameliorate apoptosis in a warm-blooded animal suffering from, or susceptible to, a neurodegenerative disorder. In one embodiment, ubiquinone prodrugs and delivery compositions are administered to a biological preparation to ameliorate apoptosis in a warm-blooded animal suffering from, or susceptible to, a neurodegenerative disorder. The association of apoptosis with a particular neurodegenerative disorder may be determined by establishing, for example, indicators of energetic defects using magnetic resonance imaging (MRI) and other standard imaging methods. Such neurodegenerative disorders include, by way of example, Parkinson's disease (Beal et al., TINS 16(4):125, 1993; Bloem et al., J. Neurol. Sci. 97:293 1990; Brennan et al., J. Neurochem. 44:1948, 1985); Alzheimer's disease (Beal et al., TINS 16(4):125, 1993; Beal, Ann. Neurol. 31:119, 1992); Huntington's disease (Beal et al., TINS 16(4):125, 1993; Bloem et al., J. Neurol. Sci. 97:293 1990; Brennan et al., J. Neurochem. 44:1948, 1985); cerebellar degenerations (Beal et al., TINS 16(4):125, 1993); and familial amyotrophic lateral sclerosis (FALS) (Olanow, TINS 16:439, 1993).

In another aspect of the present invention, ubiquinone prodrugs or delivery compositions may be administered to ameliorate apoptosis in a warm-blooded animal suffering from, or susceptible to, a late stage cardiovascular disease. The association of apoptosis with a particular late stage cardiovascular disease may be determined by any one of several means, including, by way of example, blood chemistry profiles and image analysis following injectable dyes. Such cardiovascular diseases include, by way of example, acute (intravenous) therapy for myocardial infarction, in which ubiquinones decrease apoptosis of cardiac cells following reperfusion.

In another aspect of the present invention, ubiquinone prodrugs and delivery compositions may be administered to ameliorate apoptosis in warm-blooded animals suffering arterial intima thickening associated with arteriosclerotic lesions (Schwartz et al., Diabetes Care 15:1156, 1992; Bjorkerun et al., Arterioscler. Thromb. 14:644, 1994). Thus, by way of example, the ubiquinones are administered in the treatment of arteriosclerosis and nonarteriosclerotic conditions resulting from, in part, progressive intimal thickening with age and balloon angioplasty. In a preferred embodiment, ubiquinones are stereotactically delivered to the lesion either during or immediately following balloon angioplasty.

In another aspect of the present invention, ubiquinone prodrugs or delivery compositions may be administered to ameliorate apoptosis in a warm-blooded animal suffering from or susceptible to inflammatory joint disorders. The association of apoptosis to a particular inflammatory joint disorder may be established by measurement of serum glutamic oxaloacetic transaminase (SGOT) levels, x-ray analysis of infected joints, ^{99m}Tc gamma scintigraphic imaging, arthritis score, measurement of plasma superoxidedismutase activity, and mitogenic and phagocytic responses of peripheral blood leukocytes (Miesel and Haas, Inflammation 17(5):595, 1993). Further evidence of inflammation may be obtained by NMR and depletion of key antioxidants within the synovial fluid (Fairburnet al., Clin Sci. 83(6):6570, 1992). By way of example, in the case of rheumatoid arthritis (RA), leukocytes activated by a variety of cytokines are induced to release their cytotoxic contents (NO, O_2, H_2O_2). These free radicals or oxidants destroy healthy tissues at the site of inflammation (Heliovaara et al., Ann. Rhem. Dis. 53(1):51, 1994).

In another aspect of the present invention, ubiquinone prodrugs or delivery compositions may be administered to ameliorate apoptosis in a warm-blooded animal which has been subjected to injury. Within the context of the present invention, the term "injury" refers to cellular or tissue injuries caused by physical or chemical means and, which may, ultimately result in cell death. Such injuries include, by way of example, ischemia, reperfusion, muscular degeneration, cataracts, hepatotoxicity, pancreatitis, renal injury, lung insults, brain trauma, toxic damage to liver, angina and cerebral infarction, chemotherapeutic tissue damage and accelerated aging (Linnik et al., Stroke 24(12):2002, 1993). These injuries can be identified by any one of several suitable means including standard imaging techniques or standard techniques involving measurement of lipid peroxidation products on blood plasma samples, such as that described by Satoh (Clin. Chem. Acta. 90:37, 1978). Additional tests involve the use of thiobarbaturic acid (TBARS) colorimetric assays that evaluate plasma concentration of lipid peroxide and serum levels of free radical scavenging enzymes, manganese, superoxide dismutase and catalase (Pippenger et al., Idiosyncratic Reactions to Valproate: Clinical Risk Patterns and Mechanisms of Toxicity, Levy and Penry (eds.), Raven Press, NY, Ch. 12, p. 75-88, 1991).

In yet another aspect of the present invention, ubiquinone prodrugs or delivery compositions may be administered to ameliorate apoptosis in a warm-blooded animal which has been administered therapeutics which generally result in oxidative stress. Therapeutics which cause oxidative stress may be identified by TBARS or SGOT levels as described above. Such therapeutics include, by way of example, AZT, anthracyclins, bleomycin, procarbazine, vincristin cyclophosphamide, and mitomycin-c.

The ubiquinone prodrugs and compositions of this invention may be administered to ameliorate the toxicity of chemotherapeutic agents such as adriamycin and treat periodontal

disease. The ubiquinone prodrugs and compositions may also be administered in combination with other therapeutic agents such as captopril, carnitine, tocopherol and its derivatives, and nicotinamide. The ubiquinone prodrugs and compositions of this invention may be administered in drinking water.

The ubiquinone prodrugs and compositions of the present invention are administered in a therapeutically effective dose. A therapeutically effective dose may be determined by *in vitro* experiment followed by *in vivo* studies. Based on normal plasma levels of between 0.8 and 1.0 ug/mL CoQ10, suitable dosages should normally be sufficient to raise plasma values of the ubiquinone to at least this level and, preferably, between 1 to 5 times normal. Such dosages would generally be in the range of about 10 to 400 mg of CoQ10 per day; typically be in the range of about 25 to 150, and preferably be in the range of about 30 to 100. One of ordinary skill in the art will appreciate that dosage will depend on the bioavailability of CoQ10 achieved by a particular delivery system.

Once the ubiquinone prodrugs and compositions of the present invention have been prepared as described above, they are formulated into a human or animal pharmaceutical formulation, and incorporated in an admixture with a pharmaceutical carrier and/or diluent according to conventional pharmaceutical techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, *e.g.*, intravenous, oral, topical, aerosol, inhalant, parenteral or spinal injection. The ubiquinone prodrugs and compositions are useful in the manufacture of medicaments for treating apoptosis.

In preparing the compositions in oral dosage form, any of the usual pharmaceutical medium can be employed such as, for example, water, glucose, oils, alcohols, flavoring agents, preservatives, coloring agents, or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. The aqueous solution may then be inserted, by any one of several suitable means including injection, into a suitable capsule, including, by way of example, a gel capsule, for oral administration.

In the case of parenterals, the carrier will usually comprise sterile water, though other ingredients, for example, for preservative purposes may be included. Injectable suspensions may also be prepared, in which case appropriate liquid carriers such as suspending agents, pH adjusting agents, isotonicity adjusting agents and the like may be employed.

There are unique considerations in the treatment of the central nervous system. Unlike other tissues, brain tissue is not redundant. It is highly differentiated, compartmentalized, and irreplaceable. Thus, neuropharmaceutics must be found non-toxic to normal tissues. However, it has been difficult to find the most efficacious route circumventing the blood-brain barrier. One way to bypass the barrier completely is by intracerebral spinal fluid administration by lumbar puncture or by the intraventricular route. Catheterization using the aommaya reservoir is used, but logistics dictate that to be a last resort. However, from a

practical perspective, drugs that have their site of action in the brain should in general enter the brain across the blood-brain barrier. One approach is transient reversible modification of the blood-brain barrier itself. This may be accomplished in any one of several ways, including osmotic opening or metrozol opening.

The first method is based on increasing capillary permeability by osmotically induced shrinkage of the endothelial cells which cause the widening of the intercellular-type junctions. The osmotic load is generally a hyperosmotic water-soluble agent such as mannitol or arabinose. Briefly, under general anesthesia, a transfemoral catheter is introduced into the internal carotid or vertebral artery and 150-300 mL infusion of 25% mannitol is administered at 6-10 mg/sec for thirty seconds. The intravenous infusion of the compounds of the present invention is begun approximately 5-7 minutes before the mannitol infusion and is continued for fifteen minutes. The transfemoral catheter is removed and the patient observed for 24-48 hours.

A less disruptive technique that allows enhanced drug delivery to the brain includes increasing the lipophilicity of the drug. This may be accomplished using any one of several means including, by way of example, the water soluble ubiquinone prodrugs which are cleaved to ubiquinone after passing the blood-brain barrier. In a preferred embodiment, these prodrugs are targeted to carrier/receptor systems present at the blood-brain barrier (passive drug targeting). In a particularly preferred embodiment, these prodrugs are targeted to use upregulated transport systems at the blood-brain barrier in disease states (active drug targeting).

In another embodiment, a ubiquinone prodrug may be targeted for the hexose transport system, the monocarboxylic acid transporter, neutral, basic and acidic amino acid transporters, nucleoside transporters, thiamine transporter and thyroid (T3) transport system. An example of carrier-mediated transport is L-dopa which is transported into the CNS via the neutral amino acid transporter and is then decarboxylated to yield dopamine, the active moiety. As noted above, ubiquinones can be coupled to a variety of carrier molecules including glucose lactate phenylalanine, lysine, glutamate choline thiamine, a purine or amine. Once in the CNS, the ubiquinone prodrug is cleaved to ubiquinone by esterases.

The water soluble ubiquinone derivatives of this invention include ubiquinone prodrugs that serve as effective delivery vehicles for ubiquinones such as CoQ10. As noted above, by virtue of their great water solubility, the ubiquinone prodrugs are readily formulated as aqueous solutions having high effective ubiquinone concentrations, and are thus ideal for intravenous administration. Accordingly, the ubiquinone prodrugs of this invention provide a solution to the difficulties associated with administrative routes typically required for the administration of the poorly water soluble ubiquinones including CoQ10.

In addition to being effectively and efficiently administered as aqueous solutions, the ubiquinone prodrugs of this invention are readily transported throughout the body of a warm-blooded animal and furthermore may be targeted to specific sites and tissues. The facile transport of the ubiquinone prodrugs enables delivery to various tissue sites including the brain.

The biodistribution of CoQ10 following administration of representative water soluble ubiquinone prodrugs of this invention is described in Example 15 and summarized in FIGURE 6.

The following examples are provided by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLE 1

EFFECTS OF COQ10 ON H_2O_2 TREATMENT OF CCRF-CEM CELLS

This example illustrates the effect of administering CoQ10 to CCRF-CEM cells (a T cell line) exposed to H_2O_2, a molecule capable of inducing oxidative damage and, ultimately, apoptosis in cell lines. In this example, CoQ10 is administered using a representative ubiquinone composition of the present invention. Other ubiquinones and ubiquinone prodrugs of this invention may be similarly administered.

In this experiment, three different samples of CCRF-CEM cells (a human T-cell line) were prepared by culturing a 2 mL volume of the cells (0.8×10^6) in: (1) RPMI 1640 medium and 20 uM CoQ10 in water soluble cholesterol (polyoxyethanyl-cholesteryl sebacate, Sigma Chemical Co., St. Louis, MO); (2) RPMI 1640 medium and 50 uM CoQ10 in water soluble cholesterol; and (3) RPMI 1640 medium and water soluble cholesterol. The samples were cultured for five days in plastic microtiter plates. The three samples were then further divided into two groups: those treated with 100 uM H_2O_2 for two hours and those which were not treated with H_2O_2. Cell counts were made (mean of 8 per sample), and cell death assessed by trypan blue dye exclusion at 18, 24 or 48 h after exposure to H_2O_2. The results are presented in Table 1.

TABLE 1

TREATMENT				
		H_2O_2	Cell Number ($x10^4$)	% Cytotoxicity
I. 18 h post H_2O_2	RPMI + cholesterol	-	72	0
		+	32	29.2
	20 uM CoQ10	-	140	0
		+	74	4.7
	50 uM CoQ10	-	100	0
		+	60	16.3
II. 24 h post H_2O_2	RPMI + cholesterol	-	108	0
		+	42	34.8
	20 uM CoQ10	-	178	1.7
		+	114	10.6
	50 uM CoQ10	-	188	0
		+	92	12.7
III. 48 h post H_2O_2	RPMI +cholesterol	-	150	0
		+	84	58.9
	20 uM CoQ10	-	318	1.0
		+	208	25.8
	50 uM CoQ10	-	314	3.1
		+	140	27.1

The data presented in Table 1 illustrate that the administration of CoQ10 reduced cytotoxicity against the cell line CCRF-CEM as induced by H_2O_2. In addition, at the 18 h time point, CoQ10 at a concentration of 20 uM was superior to the 50 uM concentration in mediating this effect. Finally, CoQ10 also protected living cells, as shown by the increased proliferation in the CoQ10 treated cells exposed to H_2O_2 at all time points. This observation is made by comparing the number of viable cells in the control group with the treated groups. In general, there is about a two-fold increase in the number of cells in the group receiving 20 uM CoQ10 compared to the controls.

EXAMPLE 2

EFFECTS OF CoQ10 ON UNDIFFERENTIATED NEURONAL CELLS

This example illustrates the effect of administering CoQ10 to the undifferentiated neuronal cell line PC12 (ATCC Accession Number 1721) exposed to H_2O_2. Undifferentiated PC12 cells have many of the characteristics of fully differentiated neuronal cells and thus appropriately model neuronal cell apoptosis. In this example, CoQ10 is administered using a representative ubiquinone composition of the present invention. Other ubiquinones and ubiquinone prodrugs of this invention may be similarly administered. In the following study, representative of two independent experiments, normal undifferentiated PC12 cells ($0.5X10^6$) were grown in 6 well plates containing 2 mL of RPMI medium under three different treatment conditions. In the first group (I), cells were treated for 2 h with 100 uM H_2O_2 washed and treated with fresh medium. At various time points, cells were assessed for cell death by trypan blue dye exclusion using standard techniques. In the second group (II), cells were pretreated for 2 days with solvent and a tetrahydrofuran/water soluble cholesterol/low density lipoprotein (THF/chol/LDL) mixture followed by a 2 h treatment with 100 uM H_2O_2. Next the cells were washed and fresh medium added for an additional 48 h incubation. In the third group (III), cells were pretreated for 2 days with 50 uM CoQ10 in THF/chol/LDL followed by a 2 h treatment with 100 uM CoQ10. Next the cells were washed, fresh medium containing 50 uM CoQ10 added and the cells incubated for an additional 48 h. The results are presented in Table 2.

TABLE 2

	# viable cells ($X10^4$)		% dead cells
I. Control	0 hour	149	10.0
	12 hour	44	44.0
	24 hour	13	78.0
	48 hour	35	76.5
II. Solvent	0 hour	217	10.5
	12 hour	74	23.5
	24 hour	46	56.0
	48 hour	46	66.0
III. 50 uM CoQ10	0 hour	239	7.0
	12 hour	142	14.5
	24 hour	79	40.5
	48 hour	130	40.5

The data presented in Table 2 indicate that CoQ10 reduces cytotoxicity of the undifferentiated cell line PC12 exposed to H_2O_2. The level of inhibition was approximately 50% as that observed in the control groups at the 12 h time point. In addition, CoQ10 was effective in protecting living cells from cell death at all time points tested. Furthermore, these data suggest that CoQ10 may be effective in protecting neuronal cells from oxidative stress-induced apoptosis in neurodegenerative diseases.

EXAMPLE 3

GENERAL METHODOLOGY UTILIZED IN EVALUATION OF UBIQUINONE DELIVERY COMPOSITIONS

This example serves to set forth the general methodology utilized to prepare and administer representative ubiquinone delivery compositions of the present invention. The use and effectiveness of these ubiquinone delivery compositions are described in Examples 4 to 8, which follow. In this example, CoQ10 delivery compositions, representative of the ubiquinone delivery compositions of this invention, are prepared and administered. Other ubiquinones and ubiquinone prodrugs of this invention may be similarly formulated and administered. Lipofection was utilized to deliver CoQ10 in an *in vitro* model systems designed to measure the effects of the drug on stress induced cellular apoptosis. In these studies, CoQ10 and polyoxyethanyl-cholesterol sebacate ("cholesterol", Cat. No. C1145, Sigma Chemical Co., St. Louis, MO) were solubilized in tetrahydrofuran ("THF") at a ratio of 1 mg of CoQ10 to 4 mg of cholesterol (molar ratio of approximately 1:3) and then diluted with water. The solution was evaporated to dryness under nitrogen. Next, the CoQ10-cholesterol composition was reconstituted to the appropriate volume in phosphate buffered saline (PBS) to give, for example, a stock solution of 2.0 mM or a final working solution of 50 uM.

Lipofection of various cell lines was accomplished by culturing cells with Lipofectamine™ (a 3:1 mixture of the polycationic lipid, 2,3-dioleyloxy-N-[-2-(sperminecarboamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate, "DOSPA," and the neutral lipid, dioleoyl phosphatidylethanolamine, "DOPA," in water, Gibco BRL, Gaithersberg, MD) mixed with cholesterol or cholesterol/CoQ10 prepared as described above. In a 50 uM preparation of CoQ10, for example, (25 uM cholesterol vehicle control), 25 ul of the 2 mM CoQ10 stock solution was combined with 10 ul of Lipofectamine™ and 165 ul of RPMI. The mixture was rotated in the dark for 2-3 h at room temperature to provide a liposome/CoQ10 mixture. Next the liposome/CoQ10 mixture was combined with 800 uL of a cell suspension (approximately 4 X 10^6 cells/mL). The cells are allowed to incorporate liposomes for 18 h at 37°C. Next the cells are centrifuged and washed 3 times in

RPMI/cholesterol (25 uM cholesterol). The cells are plated in 6 well tissue culture plates at 0.2 -0.5 X 10^6 cells/ 3 wells).

Induction of apoptosis was accomplished by treating the cells for various periods of time with hydrogen peroxide H_2O_2 (50 or 100 uM). Inactivation of the H_2O_2 and cessation of the oxidative insult was accomplished by adding 50 uM catalase to the cultures. This was an appropriate model for induction of oxidative stress as macrophage generate H_2O_2 during activation that can occur in a variety of conditions such as, for example, inflammation.

Cell viability was determined using a standard MTT [3-(4,5-dimethythiazol-2-yl) 2,5-diphenyltetrazolium bromide] assay (Hansen et al., J. Immun. Methods 119:203-210, 1989). This assay was based on the conversion of the tetrazolium salt to the colored product, formazan. The reaction was monitored spectrophotometrically at 570 nm. Formation of formazan was believed to occur via functional mitochondria and therefore can be an indicator of cell growth or cell death. Values were obtained by assessing absorption of 3 samples and taking the mean value.

Evaluation of ATP content of cells was accomplished using the methods of high pressure liquid chromatography (HPLC). Briefly, 2-10X10^6 cells were pelleted and lysed with 30-500 uL of ice cold water 10% TCA (trichloroacetic acid, Sigma Chem. Co., St. Louis, MO). The lysate was centrifuged in a bench top centrifuge at maximum speed. The TCA supernatant was neutralized with 200-300 uL of an ice cold mixture of tri-n-octylamine (Sigma Chemical Co., T-8631)/1,1,2-trichlorotrifluoroethane (Sigma Chemical Co., T- 9159) 1:3 v/v (*see* James et al., Cell Prolif. 27:395-406, 1994). The sample was vortexed for 30 seconds and centrifuged for 1 minute to achieve phase separation. Aliquots, 10-20 uL, of the upper phase were injected onto a SynChropac RT-P (octadecyl) reverse phase column (4.6 mm X 25 cm, Synchrom, Inc., Lafayette, IN). Elutions were carried out under isocratic conditions with a flow rate of 1 mL/min. of 1.5% acetonitrile in 0.1 M triethylammonium acetate, pH 6.8. ATP was detected at 260 nm and its content was estimated from the absorption of a known standard. Results are expressed as ng of ATP per mg of cellular protein.

Cell lines used in these studies (see following Examples 4 to 8) include CEM (ATCC accession number CCL119), a T lymphoblastoid line, established from a patient with acute lymphoblastoid leukemia; IMR-32 (ATCC accession number CCL127), a human neuroblastoma cell line, established from an abdominal tumor mass of a Caucasian male; and Neo and bcl-2 transfected cells were obtained from John Reed (Scripps Research Foundation, La Jolla, CA). CEM cells were transfected with the amphotrophic retroviruses Zip-Neo and Zip-bcl-2 as described in Miyashita and Reed (Cancer Research 52:5407-5411, 1992).

EXAMPLE 4

COMPARISON OF ANTI-APOPTOTIC EFFECT OF COQ10 VERSUS THAT OF BCL-2

This example serves to compare the ability of CoQ10 to ameliorate cell death with that of the gene bcl-2. In this example, CoQ10 is administered using a representative ubiquinone composition of the present invention. Other ubiquinones and ubiquinone prodrugs of this invention may be similarly administered.

The gene bcl-2 is a well-characterized gene that participates in the cell death pathway. The bcl-2 gene encodes a 25 kD protein that associates with membranes by a C-terminal anchor domain has the novel property, among proto-oncogenes, of inhibiting apoptosis and some forms of necrosis (Hockenbery et al., Nature 384:334-336, 1990). Cells transfected with the bcl-2 gene are protected from oxidative stress and provide a standard against which the protective effects of ubiquinones can be assessed.

In this example, CEM cells were transfected with bcl-2 gene under the control of a strong promoter (CEM-bcl-2 cells) or were transfected with a vector containing the neomycin resistant gene alone (CEM-neo cells). The CEM cells were then lipofected, as described above in Example 3, for 18 h with either Lipofectamine™ and water soluble cholesterol (25 uM) or Lipofectamine™, and a ubiquinone delivery composition comprising water soluble cholesterol and CoQ10 (50 uM) prepared as described in Example 3. The cells were aliquoted into 6-well tissue culture plates at $2X10^5$ cells/mL per well in RPMI medium.

Next the cells were washed, replated, and treated with different concentrations of H_2O_2 for 30 minutes at 37°C. After the 30 minute exposure period, the cultures were treated with catalase (50U/ml) to inactivate the H_2O_2, washed, and cultured for 18 h before analyzing cell viability by performing the MTT assay as described in Example 3. The results are summarized in Table 3.

TABLE 3: COMPARISON OF THE PROTECTIVE EFFECTS OF BLC-2 AND COQ10 IN LYMPHOID CELLS

	CEM-neo Cells		CEM-bcl2 Cells	
	Cells Surviving (%)			
H_2O_2 (μM)	Chol	CoQ10	Chol	CoQ10
0	100	100	100	100
2	95.2	96.6	92.8	99.4
5	89.3	96.1	93.0	98.3
10	84.8	93.7	94.5	94.5
20	68.6	80.3	82.6	88.0

30	57.6	72.7	81.7	80.7
40	54.6	67.0	65.4	67.8
50	48.0	60.1	57.7	65.8
100	42.8	52.8	54.9	66.6
	[n=4]	[n=5]	[n=3]	[n=4]

The data in Table 3 indicate that at all concentrations of H_2O_2 exposure examined, bcl-2 expression significantly increased cell survival. In addition, cells treated with CoQ10 showed similar protection against the oxidative effects of H_2O_2 when compared to the vehicle (cholesterol) control. These data indicate that CoQ10 treatment is as effective in protecting CEM cells from oxidative stress as is the bcl-2 gene product, and that CoQ10 serves to ameliorate oxidative stress-induced apoptosis.

In addition, the data suggests additive protection where the bcl-2 gene product is present and the cells are treated with CoQ10. Such data indicates that combination therapy which combines apoptotic inhibitors, for example, the bcl-2 gene product and a ubiquinone, is effective and provides enhanced protection to cells.

EXAMPLE 5

EVALUATION OF THE PROTECTIVE EFFECT OF COQ10 AGAINST APOPTOSIS IN IMR-32 CELLS

This example serves to demonstrate the protective effects of CoQ10 against H_2O_2-induced cell death in IMR-32 cells (ATCC accession number CCL127). In this example, CoQ10 is administered using a representative ubiquinone composition of the present invention. Other ubiquinones and ubiquinone prodrugs of this invention may be similarly administered.

In this example, IMR-32 cells were lipofected, as described above in Example 3, with either Lipofectamine™ and water soluble cholesterol (50 uM) or Lipofectamine™, and a ubiquinone delivery composition comprising water soluble cholesterol and CoQ10 (50 uM) prepared as described in Example 3, for either 18 h (Table 4) or 10 h (Table 5). Next, the cells were trypsin released, washed in fresh medium and replated in 6-well tissue culture plates at 0.2 X 10^6 cells/well and in 3 ml of RPMI. The cells were then allowed to reattach for either 2 h (Table 4) or for 12 h (Table 5) before 100 uM H_2O_2 was added to each well. After one hour, the medium was changed and cell viability was assessed using trypan blue dye exclusion. In addition, the CoQ10 content of the control and CoQ10 lipofected cells was assessed by HPLC using organic extraction procedures followed by HPLC analysis using a Altrex, ultrasphere-ODS, 5 micron particle size, 4.6 mm X 25 cm column.

The results presented in Table 4 and Table 5 indicate the CoQ10 treatment protects the cells from H_2O_2-induced apoptosis. This observation is supported in the increased

numbers of cells and in the percentage of viable cells defined by trypan blue exclusion. Also noteworthy was the highly significant uptake of CoQ10 in the treated cells. By comparison, lymphocyte cell lines generally show only about a five-fold increase in levels of CoQ10 using the same lipofection procedure, and is due at least in part to the high lipid content of the neuronal cells.

TABLE 4: NEURONAL IMR-32 CELLS:
PROTECTIVE EFFECT OF CoQ10 AGAINST H_2O_2 INDUCED CELL DEATH

	Chol		CoQ10	
	cell number ($\times 10^3$)	dead cells (%)	cell number ($\times 10^3$)	dead cells (%)
24h	116	31	275	8
48h	160	33	543	1
72h	273	46	467	9

NOTE: CoQ10 content: Control - 2.2 ng/10^6 cells, Lipofected - 396.0 ng/10^6 cells

TABLE 5: NEURONAL IMR-32 CELLS:
PROTECTIVE EFFECT OF CoQ10 AGAINST H_2O_2 INDUCED CELL DEATH

	Chol		CoQ10	
	cell number ($\times 10^3$)	dead cells (%)	cell number ($\times 10^3$)	dead cells (%)
24h	170	45	185	31
48h	350	35	590	18
72h	585	20	895	12

NOTE: CoQ10 content: Control - 2.3 ng/10^6 cells; Lipofected - 164.0 ng/10^6 cells

EXAMPLE 6

EVALUATION OF THE PROTECTIVE EFFECT OF CoQ10 AGAINST APOPTOSIS IN HUMAN T CELLS

This example serves to illustrate the protective effects of CoQ10 provides from apoptosis induced by topoisomerase inhibitor VM-26 in human T cells. In this example, CoQ10 is administered using a representative ubiquinone composition of the present invention. Other ubiquinones and ubiquinone prodrugs of this invention may be similarly administered.

VM-26 is an epipodophyllotoxin believed to inhibit topoisomerase II, thereby preventing cells from completing S phase or undergoing chromosome segregation at mitosis. VM-26 has been found to induce apoptosis in thymocytes (Walker et al. 1991). This experiment used 8E5 cells, a T lymphoblastic cell line and a derivative of CEM that contain a single defective proviral genome of HTLV III (HIV, LAV), with A3.01 cells, a T lymphoblastic cell line not infected with HTLV III, as a control. In this experiment, the T lymphoblastic cells were lipofected, as described above in Example 3, with either Lipofectamine™ and water soluble cholesterol (50 uM) or Lipofectamine™, and a ubiquinone delivery composition comprising water soluble cholesterol and CoQ10 (50 uM) prepared as described in Example 3 for 18 hours. The following day, the cells were treated with VM-26. A stock solution of 15 nM VM-26 (Bristol Laboratories, Candiac, Quebec) was prepared in ethanol and stored at -20°C. The stock solution was diluted in RPMI to give appropriate concentrations. The cells were cultured in the presence of VM-26 for 20 hours, and cell viability was tested using the MTT assay as described in Example 3. The data presented in Table 6 indicate that viability is enhanced in CoQ_{10} treated A3.01 cells compared to control cultures when treated with VM-26. However, the viability was not significantly affected in the 8E5 cells, cells infected by HIV-1. The CoQ10 content of the two cell lines was similar as shown in Table 6 indicating that differences in viability were not due to inability to deliver CoQ_{10} to the cells.

TABLE 6: HUMAN T CELLS:
PROTECTIVE EFFECT OF CoQ10 AGAINST VM-26 INDUCED CELL DEATH

	A3.01 Cells		8E5 Cells	
VM-26 (uM)	Cholesterol	CoQ_{10}	Cholesterol	CoQ_{10}
	Cells Surviving (%)			
0	100	100	100	100
0.1	91.7	99.3	88.9	94.8

0.25	87.7	95.3	70.0	59.4
0.5	74.6	90.9	48.1	46.5
1.0	69.0	85.8	33.3	38.9
2.5	49.9	91.9	18.9	30.1
5.0	39.3	97.2	24.3	28.3
CoQ_{10} content (ng/ug protein)	0.12	1.30	0.20	1.11

EXAMPLE 7

EVALUATION OF A UBIQUINONE DELIVERY COMPOSITION FOR ATP PRODUCTION

This example serves to evaluate the level of ATP in various cells following lipofection with a representative ubiquinone delivery composition of the present invention.

In addition to being an effective anti-oxidant, CoQ10 is an essential component of oxidation phosphorylation. In this example, the levels of cellular ATP are evaluated for various cells after CoQ10 administration using a ubiquinone delivery composition of this invention. Other ubiquinones and ubiquinone prodrugs of this invention may be similarly administered.

In one experiment, neuronal IMR-32 cells were lipofected, as described above in Example 3, with either Lipofectamine™ and water soluble cholesterol (50 uM) or Lipofectamine™, and a ubiquinone delivery composition comprising water soluble cholesterol and CoQ10 (50 uM) prepared as described in Example 3. After three time points, 24, 48, and 72 h, cellular ATP content was evaluated as ng/ug of cellular protein. The results shown in Table 6 indicate there was a significant increase in ATP levels in cells treated with the CoQ10 compared to the vehicle control (Chol). However, the increased levels deteriorate quickly and are absent by 72 hours.

TABLE 7

NEURONAL IMR-32 CELLS: ATP CONTENT

	Chol	CoQ10
24 h	7.3	14.6
48 h	11.4	12.6
72 h	11.4	12.0
ng/ug of protein		

In another experiment, the ATP content of human T lymphoblastic cells A3.01 and 8E5 was evaluated after treatment with CoQ10. In this experiment, the T lymphoblastic cells were lipofected, as described above in Example 3, with either Lipofectamine™ and water soluble cholesterol (50 uM) or Lipofectamine™, and a ubiquinone delivery composition comprising water soluble cholesterol and CoQ10 (50 uM) prepared as described in Example 4 for 18 hours. The following day, the cells were treated with rotenone, an uncoupler of oxidative phosphorylation between NADH and ubiquinone. Rotenone was dissolved in dimethylsulfoxide at a concentration of 1.0 mg/mL and diluted in RPMI to provide a solution having a final concentration of 5 ug/mL. Levels of ATP were assayed as ng/ug protein as described in Example 3 at the beginning of each experiment and at the 6 hour time point. The results summarized in Table 8 indicate that the ATP levels are significantly enhanced in the A3.01 cells treated with CoQ10 compared to the vehicle control (Chol). The CoQ10 content of the two cell lines was determined as described in Example 3 and was found to be similar indicating that differences in ATP content were not due to inability to deliver CoQ_{10} to the cells.

TABLE 8

HUMAN T CELLS A3.01 AND 8E5 : ATP CONTENT

ATP (ng/ug protein)	A3.01 Cells		8E5 Cells	
Time	Chol	CoQ_{10}	Chol	CoQ_{10}
0	11.1	13.1	12.0	12.4
6h	7.5	20.2	16.6	18.8
CoQ_{10} (ng/ug protein)				
	0.12	1.30	0.2	1.11

EXAMPLE 8

EVALUATION OF THE PROTECTIVE EFFECTS OF CoQ10 AGAINST STROKE USING A UBIQUINONE DELIVERY COMPOSITION

This example serves to evaluate the protective effects of CoQ10 against stroke utilizing a rat model. In this example, CoQ10 is administered using a representative ubiquinone composition of the present invention. Other ubiquinones and ubiquinone prodrugs of this invention may be similarly administered.

Brain ischemia was induced in six 340-420 g male Sprague-Dawley rat by two vessels occlusion as described by Preston et al. (Neuroscience Letters 149:75-78, 1993). Animals were pretreated with 0.3 mg/kg of atropine sulfate and anesthetized with 60 mg/kg of sodium pentobarbital. The rats were intubated and mechanically ventilated during surgery to maintain

normal levels of blood gases. Brain ischemia was induced by reducing arterial blood pressure to 42-47 mm Hg (blood withdrawal from a tail artery) followed by bilateral occlusion of common carotid arteries using arterial clamps. At either 8 or 12 minutes post-occlusion, the blood was reinfused, the carotid clamps were removed and wounds were closed. Colonic and tympanic temperatures were monitored and regulated during surgery within 37.5-38.0°C using a water pad beneath the animal until self thermoregulation was reestablished.

A representative ubiquinone delivery composition of the present invention comprising CoQ10 and water soluble cholesterol prepared as described in Example 3 was administered to one set of rats. The ubiquinone delivery composition (effectively delivering 3 mg CoQ10/kg rat body weight) was administered twice (by intraperitoneal injection, 0.5 mL PBS containing 1 mg CoQ10 and 4 mg water soluble cholesterol per injection), once immediately after the surgery, and then 3 h later. One set of control rats where treated with 500 uM water soluble cholesterol (cholesterol control) in the same time frame, and another set of control rats were not treated (control). Following death, the rats were perfused with 4% buffered formalin, and brain tissue was removed and embedded in paraffin. Paraffin sections, 5 μm, of the brain were prepared, H/E (hematoxylin/eosin) stained and photographed.

Ischemic brain injury in this model usually leads to selective neuronal loss in stratum and particularly in hippocampus. The result is readily visualized after brain section staining with H/E. Initially (within 24 to 48 h after the ischemic episode), the affected neurons are darker, visibly shrunken and detached from neighboring cells. At later times (3 to 6 days) the cells are gone (probably being engulfed by phagocytic cells) leaving gaps or holes in brain tissue. These gaps or holes are eventually filled by gila cells leading to a severe memory loss in these ischemic animals. As can be seen in FIGURE 3, 3 days after surgery there was significant neuronal cell death (see apoptotic cells identified by 'a' in FIGURE 3) in rats treated with the vehicle control that is characteristic of the ischemia induced in this type of model system. However, in FIGURE 4, 3 days after surgery the animals treated with CoQ10 show little or no cell death (see non-apoptotic neuronal cells identified by 'b' in FIGURE 4). This observation is made more remarkable by the fact that other inhibitors of stroke only demonstrate partial protection of these neurons.

Further, as noted in Table 9, rats injected with CoQ10 had a 10-fold higher plasma level and 2-fold higher brain level of the drug when analyzed 24 h after the injections, indicating bioavailability of the drug.

TABLE 9:

COQ10 CONTENT IN RATS FOLLOWING INTRAPERITONEAL ADMINISTRATION

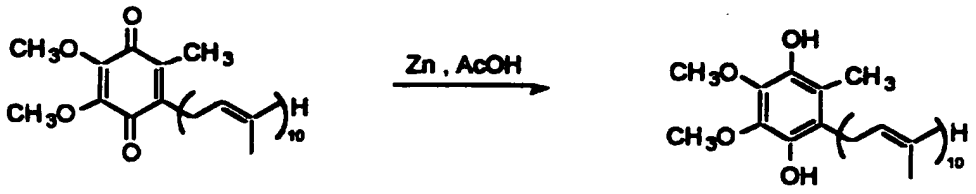
	PLASMA (ng/mL)	BRAIN (μg/g)
Control	13.6	2.47
Cholesterol Control	18.2	3.65
CoQ10/Cholesterol	196.6	7.33

FIGURE 5 summarizes the percentage of apoptotic cells for rats treated with the ubiquinone delivery composition (CoQ10 treated) and those treated with water soluble cholesterol (untreated) as a function of days after reperfusion for both the 8- and 12-minute occlusions. In the FIGURE, the percent of dead hippocampal neurons (standard error of mean) at the indicated periods following reperfusion was assessed by microscopy of fixed tissue sections and "ND" refers to "not determined".

EXAMPLE 9

REDUCTION OF UBIQUINONE TO UBIQUINOL

This example serves to demonstrate a method of reducing ubiquinone(50) to ubiquinol(50). The method is generally applicable to the reduction of the quinone group of any ubiquinone or ubiquinone derivative to its corresponding ubiquinol.



Ubiquinol was synthesized from ubiquinone according to Cheng and Casida (J. Labl. Compd. Radiopharm. 6:66-75, 1970). Briefly, a mixture of 1 g of coenzyme Q10, 5 mL of acetic acid and 0.3 g of zinc dust was heated at 50°C for 5 min., quenched with 5 mL of water and extracted in hexane (45 mL), in an argon atmosphere. Hexane extract was washed with water (3 x 5 mL), dried over $MgSO_4$, concentrated on a rotoevaporator to obtain 1.10 g of colorless viscous material which was dried at high vacuum (0.05 mm) at room temperature for 1 h to yield 0.980 g of reduced coenzyme Q10. This material was used in the following reactions without further purification.

EXAMPLE 10

SYNTHESIS OF A REPRESENTATIVE UBIQUINONE PRODRUG: CoQ10-ETHER-PEG

This example serves to demonstrate the synthesis of a representative ubiquinone prodrug of the present invention, CoQ10-ether-PEG. For this ubiquinone prodrug, the ubiquinone is CoQ10, the solubilizing moiety is polyethylene glycol 5000, an electronically neutral solubilizing moiety, and the linking group is an ether. The following procedure provides a general methodology for preparing a ubiquinone prodrug having a solubilizing moiety coupled directly to a ubiquinol with an ether linking group. Accordingly, the synthetic procedure is applicable to the ubiquinones and solubilizing moieties of this invention.

In a 250 mL three necked round bottom flask equipped with a dropping funnel and a reflux condenser, 250 mg (0.288 mmol) of ubiquinol (reduced CoQ10 prepared as described in Example 9) was dissolved in 30 mL of anhydrous tetrahydrofuran (THF)(added via a syringe) under an argon atmosphere. To this solution was added 21 mg (0.86 mmol) of sodium hydride. The reaction mixture was stirred at room temperature for 15 min., then cooled to 0°C using an ice bath. To the cooled solution was added 1.44 g (0.288 mmol) of PEG-tresylate (Aldrich Chemical Co., Milwaukee, WI) in 20 mL anhydrous tetrahydrofuran via a dropping funnel over a period of 20 min. After stirring the reaction mixture at 0°C for 30 min., the ice bath was removed and the reaction mixture was stirred for an additional 1 h. The reaction mixture was then brought to the reflux temperature by slowly raising the temperature of oil bath to 100°C. After 2 h at reflux, heating was discontinued and the reaction mixture was allowed to cool to room temperature. The reaction mixture was quenched with a mixture of brine and THF (2 mL each). The solids were vacuum filtered and solvent was evaporated on a rotoevaporator. The thick oily material obtained was dissolved in 100 mL of chloroform and washed with water (2 x 10 mL). The organics were dried over anhydrous magnesium sulfate, filtered, concentrated on a rotoevaporator, and dried under vacuum at room temperature for 2 h to yield 1.5 g of a yellow solid.

The crude product was loaded on a 2.5 x 28 cm flash silica column and eluted with chloroform as the solvent. CoQ_{10} and other related impurities were eluted with chloroform and the pegylated products were eluted with 10 % methanol in chloroform to yield 1.21 g of a faint yellow solid.

In a second purification step, 100 mg of the above chromatographed material was loaded on a 1 x 16 cm of reverse phase silica gel column. The desired mono-pegylated CoQ_{10} (designated "CoQ10-ether-PEG") was obtained in 58% yield by using methanol as the eluent. Di-pegylated CoQ10 (designated "CoQ10-(ether-$PEG)_2$) was obtained as a minor product.

Physical Data: IR (mujol, cm^{-1}): 1650, 1460, 1375, 1340, 1275, 1240, 1060, 960, 945, 840, 720. ^{1}H-NMR ($CDCl_3$, δ): 5.12 (m, vinylic-H), 4.9 (t, vinylic -H), 3.9 - 3.3 (PEG-H), 2.08 - 1.85 (m, allylic CH_2), 1.7-1.6 (m, CH_3). mp = 59 - 60 °C.

Method for Assessing Ubiquinone Prodrug Water Solubility

Water solubility of CoQ10-ether-PEG is evaluated by diluting 0.10 mg in 1.0 mL of water. The solution is stirred for 1 h and allowed to stand at room temperature for 24 h. The sample is then centrifuged and the resultant aqueous layer analyzed using high pressure liquid chromatography ("HPLC"). The HPLC analysis is conducted isocratically using acetonitrile as the solvent on a LiChrospher 100, C-18 column (5 μM, 125 x 4 mm) using a flow rate of 2 mL/min. Under these conditions, CoQ10-ether-PEG has a retention time of 7.6 min.

The quantitation of the coenzyme Q_{10}-containing product is accomplished by HPLC using UV detection at 254 nm. In the quantitation, a an aqueous solution of a coenzyme Q10 derivative is prepared and analyzed by HPLC as described above. A series of coenzyme Q10 aqueous solutions of known concentration are prepared and analyzed by HPLC. The results of these HPLC analyses are then used to construct a standard curve where the concentration of the coenzyme Q10 standard is plotted against the HPLC signal for the standard. Once such a standard curve has been constructed, aqueous solutions of coenzyme Q10 derivatives may be similarly analyzed and the concentration of the derivative in the solution determined.

EXAMPLE 11

SYNTHESIS OF A REPRESENTATIVE UBIQUINONE PRODRUG: COQ10-CARBAMATE-PEG

This example serves to demonstrate the synthesis of a representative ubiquinone prodrug of the present invention, CoQ10-carbamate-PEG. For this ubiquinone prodrug, the ubiquinone is CoQ10, the solubilizing moiety is polyethylene glycol 5000, an electronically neutral solubilizing moiety, and the linking group is a carbamate. The following procedure provides a general methodology for preparing a ubiquinone prodrug having a solubilizing moiety coupled directly to a ubiquinol with a carbamate linking group. Accordingly, the synthetic procedure is applicable to the ubiquinones and solubilizing moieties of this invention.

The following synthesis of CoQ10-carbamate-PEG is a two-step process. In the first step, the succinimidyl carbamate of amino-methoxy-PEG 5000 was prepared by dissolving 1.0 g (0.20 mmol) amino-methoxy-PEG 5000 (Shearwater Polymer, Inc., Huntsville, AL) in 15 mL dry dioxane and warming to 50°C to achieve solution. To the cooled solution was added a solution of 359 mg (1.4 mmol) N,N'-disuccinimidyl carbonate in 10 mL acetone. A solution of 171 mg (1.4 mmol) dimethylaminopyridine in 10 mL acetone was then added and the resulting reaction mixture was stirred overnight at room temperature. The crude product was precipitated from the solution by the addition of 60 mL diethyl ether, filtered, and dried at under vacuum at room temperature for 3 hours to yield 1.0 g (93%) of the succinimidyl carbamate of amino-methoxy-PEG 5000.

In a 100 mL three necked round bottom flask equipped with a dropping funnel and argon balloon was added 216 mg (0.25 mmol) of ubiquinol (reduced CoQ10 prepared as described in Example 10) in 10 mL of anhydrous tetrahydrofuran. To this solution was added 15 mg (0.62 mmol) of sodium hydride. The reaction mixture was stirred at room temperature for 5 min., then cooled to 0°C using an ice bath. To the cooled solution was added 500 mg (0.10 mmol) of the succinimidyl carbamate of amino-methoxy-PEG 5000 prepared as described above in 5 mL anhydrous tetrahydrofuran via a dropping funnel. After stirring the reaction mixture at 0°C for 20 min., the reaction mixture was quenched with a mixture of brine and THF (2 mL each), and the solvent was evaporated on a rotoevaporator. The crude product was taken up in 50 mL of chloroform and washed with water (2 x 10 mL). The organics were dried over anhydrous magnesium sulfate, filtered, concentrated on a rotoevaporator, and dried under vacuum at room temperature for 8 h to yield 0.57 g of a light yellow solid.

The crude product was loaded on a 2.5 x 28 cm flash silica column and eluted with chloroform as the solvent. CoQ_{10} and other related impurities were eluted with chloroform and the pegylated products were eluted with 50 % methanol in chloroform to yield 0.49 g of a faint yellow solid.

In a second purification step, the above chromatographed material was loaded on a 2 x 26 cm of reverse phase silica gel column. Unreacted PEG and related impurities were eluted with 70% methanol-water (1% acetic acid) and the desired mono-pegylated CoQ_{10} (designated "CoQ10-carbamate-PEG") was eluted with 100% methanol. The purified CoQ10-carbamate-PEG was obtained in 40% yield.

EXAMPLE 12

SYNTHESIS OF A REPRESENTATIVE UBIQUINONE PRODRUG: COQ2-ETHER-SULFONATE

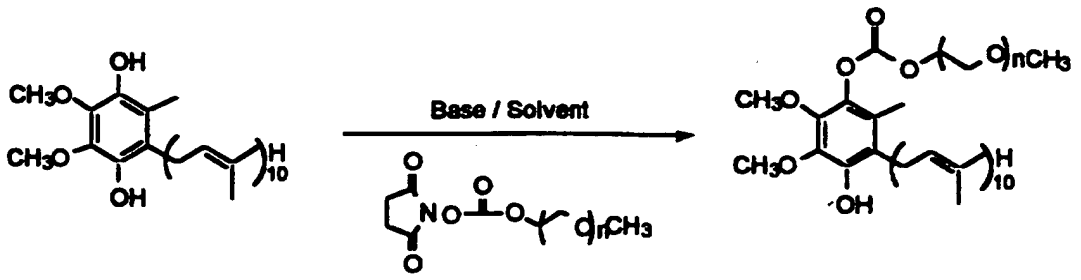
This example serves to demonstrate the synthesis of a representative ubiquinone prodrug of the present invention, CoQ2-ether-sulfonate. For this ubiquinone prodrug, the ubiquinone is CoQ2, the solubilizing moiety is a sulfonate group, an electronically charged solubilizing moiety, and the linking group is an ether. The following procedure provides a general methodology for preparing a ubiquinone prodrug having a solubilizing moiety coupled directly to a ubiquinol with an ether linking group. Accordingly, the synthetic procedure is applicable to the ubiquinones and solubilizing moieties of this invention.

To a 250 mL three neck round bottom flask containing 1.0 g (3.12 mmol) reduced CoQ2, prepared as described in Example 9, and 100 mL anhydrous THF is added 160 mg(6.7 mmol) sodium hydride. After stirring at room temperature until the evolution of gas has ceased, the reaction mixture is cooled in an ice bath and 0.75 g (3.6 mmol) sodium 2-bromoethanesulfonate (Aldrich Chemical Co., Milwaukee, WI) is added and the resulting reaction mixture heated to reflux. The progress of the reaction is monitored by HPLC. After the starting ubiquinol has been consumed, the reaction mixture is cooled to room temperature and the solvent removed under vacuum. To the crude product is added 50 mL of a water/5% acetic acid solution with stirring. The aqueous mixture is then filtered and 20 mL of 1 M tetramethylammonium chloride is added. The crude product is then extracted with diethyl ether, and the combined extracts are dried over anhydrous magnesium sulfate and concentrated to dryness. The crude product is then purified by ion exchange chromatography by elution from a sodium ion exchange column.

EXAMPLE 13

SYNTHESIS OF A REPRESENTATIVE UBIQUINONE PRODRUG: CoQ10-CARBONATE-PEG

This example serves to demonstrate a representative ubiquinone prodrug of the present invention in which the ubiquinone is CoQ10, the solubilizing moiety is a polyethylene glycol and the linking group is a carbonate. The following procedure provides a general methodology for preparing a ubiquinone prodrug having a solubilizing moiety coupled directly to a ubiquinol with a carbonate linking group. Accordingly, the synthetic procedure is applicable to the ubiquinones and solubilizing moieties of this invention.



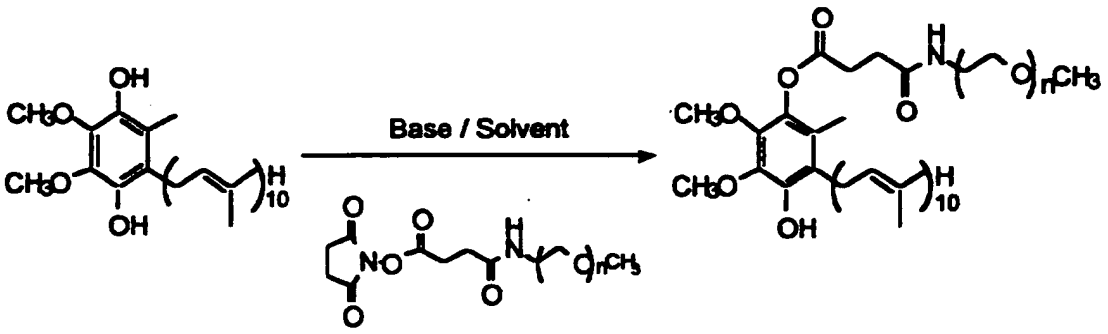
Treatment of the succinimidyl carbonate of PEG with reduced coenzyme Q_{10} in the presence of appropriate base (such as sodium hydride) in appropriate solvent (such as tetrahydrofuran).

Succinimidyl carbonate of methoxy-PEG-5000 is commercially available (Shearwater Polymers, Inc., Huntsville, AL), and may be synthesized from methoxy-PEG (*see, e.g.*, Miron and Wilchek, Bioconjugate Chem. 4:568-569, 1993; Greenwald et al., J. Org. Chem. 60:331-336, 1995). Similarly succinimidyl carbonate of methoxy-PEG of various sizes may also be synthesized in this manner. Solubility may be ascertained as outlined above in Example 10.

EXAMPLE 14

SYNTHESIS OF A REPRESENTATIVE UBIQUINONE PRODRUG: COQ10-ESTER-PEG

This example serves to demonstrate a representative ubiquinone prodrug of the present invention in which the ubiquinone is CoQ10, the solubilizing moiety is a polyethylene glycol and the linking group is an ester. The following procedure provides a general methodology for preparing a ubiquinone prodrug having a solubilizing moiety coupled directly to a ubiquinol with an ester linking group. Accordingly, the synthetic procedure is applicable to the ubiquinones and solubilizing moieties of this invention.



Treatment of reduced coenzyme Q10 with N-hydroxysuccinimidyl active ester of methoxy-PEG-succinamide (NHS Ester of PEG) of varying sizes mainly PEG-5000, PEG-2000, PEG-750 and PEG-350 in the presence of appropriate base (such as triethylamine or pyridine) in appropriate solvent (such as tetrahydrofuran). Solubility may be ascertained as outlined above in Example 10.

The N-hydroxysuccinimidyl active ester of methoxy-PEG-succinamide is commercially available (Shearwater Polymers, Inc., Huntsville, AL), it is synthesized according to a literature procedure from the amino methoxy-PEG prepared as described above in Example 11 (*see, e.g.*, Miron and Wilchek, Bioconjugate Chem. 4:568-569, 1993; Greenwald et al., J. Org. Chem. 60:331-336, 1995).

EXAMPLE 15

CoQ10 BIOAVAILABILITY AFTER ADMINISTRATION OF REPRESENTATIVE UBIQUINONE PRODRUGS AND UBIQUINONE DELIVERY COMPOSITIONS

This example serves to illustrate the bioavailability of CoQ10 following administration of representative ubiquinone prodrugs and ubiquinone compositions of the present invention. In this example, the effective equivalent of 1 mg of CoQ10 was administered to rats by intraperitoneal injection of sterile PBS solutions of (1) a representative ubiquinone delivery composition comprising CoQ10 and water soluble cholesterol prepared as described in Example 3; and representative ubiquinone prodrugs (2) CoQ10-carbamate-PEG prepared as described in Example 11, (3) CoQ10-ether-PEG prepared as described in Example 10, and (4) CoQ10-(ether-PEG)$_2$ prepared as described in Example 10.

The bioavailability of CoQ10 in tissues 24 hours after intraperitoneal injection is summarized in Table 10 (values represent the mean CoQ10 level for two rats) and FIGURE 6. The CoQ10 levels were determined by the methods described in Example 3.

TABLE 10

BIOAVAILABILITY OF CoQ10 IN RATS AFTER INTRAPERITONEAL ADMINISTRATION OF UBIQUINONE COMPOSITION AND PRODRUG

	Plasma (ng/mL)	Liver (ug/g)	Brain (ug/g)
Endogenous CoQ_{10}	50.9	5.0	8.7
CoQ_{10} delivery composition	374	36.7	11.6
CoQ_{10} - carbamate - PEG	100.5	4.5	10.3
CoQ_{10} - ether - PEG	415	6.1	10.0
CoQ_{10} - (ether - PEG)$_2$	87.6	4.3	10.9

The results indicate a significant increase in plasma levels of CoQ10 for each ubiquinone composition and prodrug administered. The data indicate that the rat is able to efficiently catabolize the administered prodrugs and render the CoQ10 thus formed available for metabolic needs.

EXAMPLE 16

MANUFACTURING PROCESS FOR THE PREPARATION OF A REPRESENTATIVE UBIQUINONE PRODRUG

This example serves to illustrate a manufacturing process for the preparation of the water soluble ubiquinone prodrugs of the present invention. While it is appreciated that the ubiquinone prodrugs can be prepared by a variety of methods including those presented in the Examples above, the following manufacturing process takes advantage of the difference in the solubility properties of the ubiquinone starting material and prodrug product to effect the separation and isolation of the prodrug process products. Generally, ubiquinones (including their corresponding ubiquinols) are highly water insoluble. In contrast, the ubiquinone prodrugs are highly water soluble.

The manufacturing process for preparing a ubiquinone prodrug from ubiquinone includes at least two steps. In the first step, a ubiquinone is reduced to its ubiquinol (*see, e.g.*, Example 9), and in a second step, a suitably reactive solubilizing moiety is coupled to the ubiquinol to form the prodrug product (*see, e.g.*, Examples 10-14). The process includes the use of organic solvents for the reduction and coupling steps. Through the use of a limiting amount of the suitably reactive solubilizing moiety, the coupling reaction will consume essentially all of the solubilizing moiety thus leaving unconverted ubiquinol in the reaction mixture. Under these conditions, the reaction mixture contains unreacted ubiquinol (or its oxidation product ubiquinone) and the water soluble prodrug. However, the use of a limiting amount of a suitably reactive solubilizing moiety is not essential.

In one embodiment, the manufacturing process is a filtration process and involves separation by filtration of the aqueous prodrug solution from the aqueous insoluble organic residue of the reaction mixture. In another embodiment, the manufacturing process is an extraction process and involves extraction of the water soluble prodrug product from the reaction mixture.

Isolation of the ubiquinone prodrugs by the filtration process involves the steps of removing the organic solvents from the reaction mixture by, for example, evaporation to provide a reaction mixture residue; adding an aqueous solvent such as water to the reaction mixture residue to dissolve the product prodrug and provide a heterogeneous product solution comprising an aqueous prodrug solution and an aqueous insoluble organic residue comprising unreacted ubiquinol and ubiquinone; filtering the heterogeneous product solution to separate

the aqueous prodrug solution from the aqueous insoluble organic residue; and concentrating the aqueous prodrug solution to dryness to provide the prodrug product.

Isolation of the ubiquinone prodrugs by the extraction process involves the steps of adding an aqueous solvent to provide a biphasic solution comprising an aqueous prodrug solution and a water-immiscible organic solution comprising unreacted ubiquinol and ubiquinone; separating the aqueous prodrug solution and the water-immiscible organic solution; and concentrating the aqueous prodrug solution to dryness to provide the prodrug product. To effect the formation of the biphasic solution, the extraction process may further include the step of adding a water-immiscible organic solvent such as hexane or diethyl ether.

EXAMPLE 17

EVALUATION OF CoQ10 AGAINST STROKE IN PRIMATES

This example serves to evaluate CoQ10 utilized against stroke in primates. For these studies global or forebrain ischemia is induced in anesthetized primates using the technique of surgical occlusion of the carotids (Sengupta et al., J. Neurol. Neurosurg. Psychiatry 36:736-741, 1973) or using the technique of 4 vessel occlusion, carotid and vertebral arteries (Wolin et al., Resuscitation 1:39-44, 1992). Both of these methods produce global forebrain ischemia with damage selectively limited to vulnerable neuronal populations. The time course over which this ischemic maturation occurs may be hours or days, depending on the duration of the ischemia and vulnerability of the neuronal population. Examples of ischemia-sensitive neurons include the hippocampal CA1 pyramidal neurons, the cerebellar Purkinje cells, striatal neurons of medium or small size and pyramidal neurons in the midneocortical layers.

Following the induction of the ischemic event, water soluble ubiquinone prodrugs or ubiquinone delivery compositions are solubilized in a solution at concentrations ranging from 50 to 200 mg/ml, is administered intravenously at various time points (*e.g.*, at time 0, 30, 60, 120, 240, and 480 minutes) in either single or multiple injections. The animals receive various doses of the ubiquinone, ranging from 50 to 300 mg. The vehicle control is also administered for comparative purposes. Following recovery from anesthesia, the animals are studied for motor and behavioral activity. In addition, memory and ability to remember new tasks are evaluated using techniques specific to the species of primate. Twelve hours to six days following the induction of the 'stroke', the animals are sacrificed and the regions of the brain sensitive to ischemic damage as defined by apoptosis are prepared using histologic techniques. The tissues are then examined microscopically for ischemic damage. Efficacy of the protective effect of ubiquinone is based on both behavior and the histopathology of the primate's neuronal tissues.

From the foregoing, it will be evident that although specific embodiments of the invention have been described herein for the purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

Claims

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A water soluble ubiquinone prodrug comprising a ubiquinol substituted at at least one of positions C1 and C4 of the ubiquinol with a substituent independently selected from a solubilizing moiety and a targeting moiety.

2. The ubiquinone prodrug of Claim 1 wherein the ubiquinol comprises a terpenoid side chain having from 2 to 12 isoprenoid units.

3. The ubiquinone prodrug of Claim 1 wherein the ubiquinol comprises a terpenoid side chain having 10 isoprenoid units.

4. The ubiquinone prodrug of Claim 1 wherein at least one substituent solubilizing moiety further comprises a targeting moiety.

5. The ubiquinone prodrug of Claim 1 wherein at least one substituent targeting group further comprises a solubilizing moiety.

6. The ubiquinone prodrug of Claim 1 wherein at least one solubilizing moiety or targeting moiety is linked to position C1 or C4 of the ubiquinol through a linking group.

7. The ubiquinone prodrug of Claim 6 wherein said linking group is selected from the group consisting of carbamate, carbonate, ether, and ester.

8. The ubiquinone prodrug of Claim 6 wherein the linking group is selected from the group consisting of carbamate and ether.

9. The ubiquinone prodrug of Claim 1 wherein said solubilizing moiety is selected from the group consisting of electronically charged and electronically neutral solubilizing moieties.

10. The ubiquinone prodrug of Claim 9 wherein said electronically charged solubilizing moiety is selected from the group consisting of sulfonate, carboxylate, phosphonate, ammonium, polyanion, polycation, and polypeptide moieties.

11. The ubiquinone prodrug of Claim 10 wherein said electronically neutral solubilizing moiety is selected from the group consisting of hydroxy, amine, thiol, polyalcohol, polyether, polyamine, polysaccharide, and polypeptide moieties.

12. The ubiquinone prodrug of Claim 11 wherein said solubilizing moiety is a polyether.

13. The ubiquinone prodrug of Claim 12 wherein said polyether is a polyethylene glycol.

14. The ubiquinone prodrug of Claim 13 wherein said polyethylene glycol has a molecular weight in the range from about 350 to about 6000.

15. The ubiquinone prodrug of Claim 14 wherein said polyethylene glycol has a molecular weight in the range from about 600 to about 3400.

16. The ubiquinone prodrug of Claim 15 wherein said polyethylene glycol has a molecular weight in the range from about 1500 to about 2500.

17. The ubiquinone prodrug of Claim 1 wherein said targeting moiety is targeted to a receptor selected from the group consisting of an LDL receptor, an asialoglycoprotein receptor, a polyamine receptor, an insulin receptor, a transferrin receptor, and an alpha-2-macroglobulin receptor.

18. The ubiquinone prodrug of Claim 17 wherein said receptor is selected from the group consisting of an asialoglycoprotein receptor and a polyamine receptor.

19. A water soluble ubiquinone prodrug comprising ubiquinone(50) linked to polyethylene glycol 5000.

20. A pharmaceutical composition comprising a ubiquinone prodrug according to any one of Claims 1-19 and optionally a pharmaceutically acceptable excipient or diluent.

21. A ubiquinone delivery composition comprising a ubiquinone and a solubilizing agent, wherein said solubilizing agent comprises a lipophilic moiety and an electronically neutral solubilizing moiety.

22. The composition of Claim 21 wherein said electronically neutral solubilizing moiety is selected from the group consisting of hydroxy, amine, thiol, polyalcohol, polyether, polyamine, polysaccharide, and polypeptide moieties.

23. The composition of Claim 22 wherein said electronically neutral solubilizing moiety is a polyether.

24. The composition of Claim 23 wherein said polyether is a polyethylene glycol.

25. The composition of Claim 24 wherein said lipophilic moiety is selected from the group consisting of lipids and phospholipids.

26. The composition of Claim 25 wherein said lipid is selected from the group consisting of fatty acids, fatty alcohols, and fatty esters.

27. The composition of Claim 26 wherein said fatty alcohol is a sterol.

28. The composition of Claim 27 wherein said sterol is a cholesterol.

29. A ubiquinone delivery composition, comprising a water soluble ubiquinone prodrug as claimed in any one of Claims 1 to 19 and a solubilizing agent, wherein said solubilizing agent comprises a lipophilic moiety and an electronically neutral solubilizing moiety.

30. The composition of Claim 29 further comprising a ubiquinone.

31. A pharmaceutical composition comprising a ubiquinone delivery composition according to any one of Claims 21 to 30 and optionally a pharmaceutically acceptable excipient or diluent.

32. A method of ubiquinone delivery, comprising administering to a warm-blooded animal a water soluble ubiquinone prodrug as claimed in any of Claims 1 to 19.

33. A method of ubiquinone delivery, comprising administering to a warm-blooded animal a ubiquinone delivery composition as claimed in any of Claims 21 to 30.

34. A method of ubiquinone delivery, comprising administering to a warm-blooded animal a pharmaceutical composition of Claim 20 or Claim 31.

35. A method of ameliorating apoptosis, comprising administering to a warm-blooded animal in need thereof a therapeutically effective amount of a ubiquinone, wherein said ubiquinone is administered as a water soluble ubiquinone prodrug as claimed in any of Claims 1 to 19.

36. The method of Claim 35 wherein the apoptosis is associated with ischemia, a viral disorder, or a neurodegenerative disorder.

37. A method of ameliorating apoptosis, comprising administering to a warm-blooded animal in need thereof a therapeutically effective amount of a ubiquinone, wherein

said ubiquinone is administered as a ubiquinone delivery composition as claimed in any of Claims 21 to 30.

38. The method of Claim 37 wherein the apoptosis is associated with ischemia, a viral disorder, or a neurodegenerative disorder.

39. A method of ameliorating apoptosis, comprising administering to a warm-blooded animal in need thereof a therapeutically effective amount of a ubiquinone, wherein said ubiquinone is administered as a pharmaceutical composition as claimed in Claim 20 or Claim 31.

40. The method of Claim 39 wherein the apoptosis is associated with ischemia, a viral disorder, or a neurodegenerative disorder.

41. The use of a solubilizing moiety linked to at least one of positions C1 and C4 of a ubiquinol to increase the *in vitro* aqueous solubility of a ubiquinone representing an oxidized form of the ubiquinol.

42. The use of a ubiquinol linked through at least one of its positions C1 and C4 to a solubilizing moiety to increase the *in vitro* aqueous solubility of a ubiquinone.

43. The use of a water soluble ubiquinone prodrug of any of Claims 1 to 19, a pharmaceutical composition of Claim 20 or Claim 31, or a ubiquinone delivery composition of any of Claims 21 to 30 in the manufacture of a medicament for treating by prophylaxis or therapy one or more conditions selected from among: (i) apoptosis; (ii) apoptosis associated with ischemia; (iii) apoptosis associated with a viral disorder; and (iv) apoptosis associated with a neurodegenerative disorder.

44. A pharmaceutical composition for ameliorating apoptosis, comprising a therapeutically effective amount of a ubiquinone, wherein said ubiquinone is a administered in a form selected from a water soluble ubiquinone prodrug as claimed in any of Claims 1 to 19 and a ubiquinone delivery composition as claimed in any of Claims 21 to 30.

45. The pharmaceutical composition of Claim 44 wherein apoptosis is associated with ischemia, a viral disorder, or a neurodegenerative disorder.

46. A method for manufacturing a ubiquinone prodrug comprising, reducing a ubiquinone to form a ubiquinol, coupling a solubilizing moiety to the ubiquinol to form a ubiquinone prodrug, and isolating the ubiquinone prodrug.

47. The method of Claim 47 wherein isolating the ubiquinone prodrug comprises the steps of removing the solvent from a ubiquinone prodrug reaction mixture to provide a reaction mixture residue, adding an aqueous solvent to the reaction mixture residue to provide a heterogeneous product solution comprising an aqueous prodrug solution and an insoluble residue, filtering the heterogeneous product solution to effect separation of the aqueous prodrug solution from the insoluble residue to provide an aqueous prodrug solution, and concentrating the aqueous prodrug solution to dryness to provide the ubiquinone prodrug.

48. The method of Claim 47 wherein isolating the ubiquinone prodrug comprises the steps of adding an aqueous solvent to the ubiquinone prodrug reaction mixture to provide a biphasic solution comprising an aqueous prodrug solution and a water-immiscible solution, separating the biphasic solution to effect separation of the aqueous prodrug solution and the water-immiscible solution to provide the aqueous prodrug solution, and concentrating the aqueous prodrug solution to dryness to provide the ubiquinone prodrug.

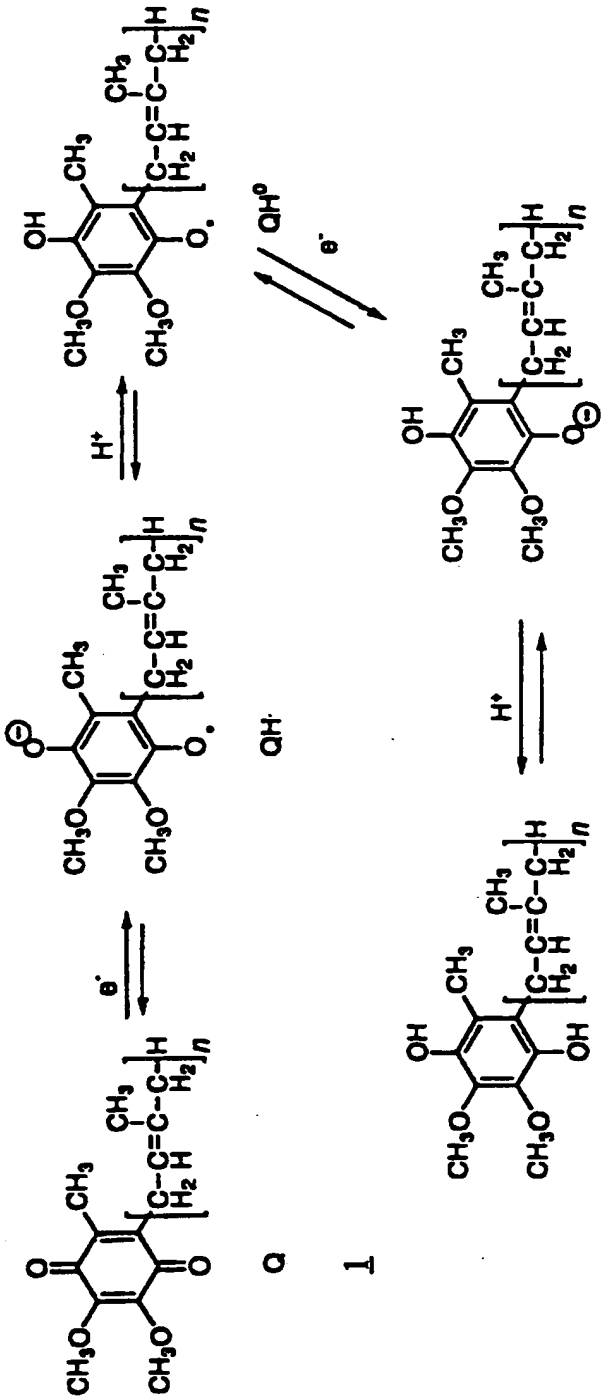


FIGURE 1.

"X+" / Base
Carbamate Derivatives
X = —C(=O)—N(H)—R
Alkyl Ether Derivatives
X = —CH2R
Ester Derivatives
X = —C(=O)—R
Carbonate Derivatives
X = —C(=O)—O—R

FIGURE 2.

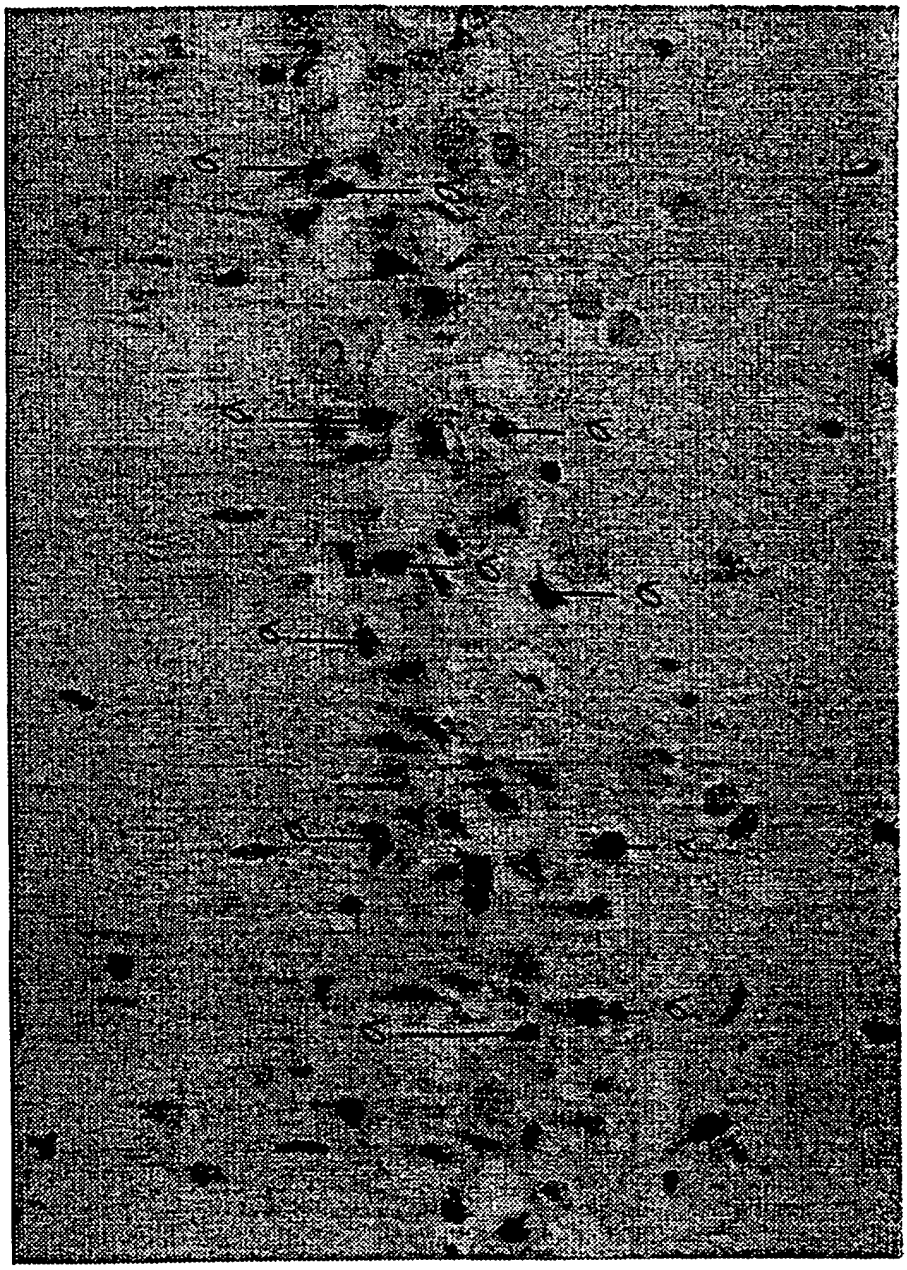


Figure 3.

Figure 4.

FIGURE 5.

PERCENT APOPTOTIC CELLS

	Days After Reperfusion				
12-Minute Occlusion	1	2	3	6	12
Untreated Rats	13 (3.5)	100 (0)	100 (0)	100 (0)	ND
CoQ10 Treated Rats	2 (0.9)	2 (0.9)	60 (14)	85 (3.5)	ND
8-Minute Occlusion					
Untreated Rats	ND	ND	70 (4)	85 (3.5)	100 (0)
CoQ10 Treated Rats	ND	ND	1 (0.2)	50 (6)	54 (10)

FIGURE 6.

CoQ_{10} Source	Plasma (ng/ml)	Liver (ug/g)	Brain (ug/g)
Endogenous CoQ_{10}	50.9	5.0	8.7
Prodrug CoQ_{10}			
CoQ_{10} - carbamate - PEG	100.5 (+97%)	4.5	10.3 (+18%)
CoQ_{10} - ether - PEG	415 (+715%)	6.1	10.0 (+15%)
CoQ_{10} - (ether - PEG)$_2$	87.6 (+72%)	4.3	10.9 (+25%)